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Re-evaluation of role of Stem fly, *Melanagromyza sojae* (Zehntner) (Diptera : Agromyzidae) in soybean

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Abstract

An experiment was conducted in Spring season with soybean variety Bragg which was sown in two separate 5 x 5 mt plots One plot was maintained as untreated plot while second plot was treated with phorate (10 G) @ 10 kg/ha at the time of sowing and three times as side dressing at 30 days interval Leaf feeders were destroyed manually at regular intervals From each plot (treated and untreated) 100 plants were randomly selected and uprooted during harvesting Observations were recorded on tunnel length in main stem, branches and whole plant as well as yield parameters Correlation was computed between tunnel length in main stem, branches and whole plant with yield parameters. Plants obtained from untreated and treated plots failed to show any significant correlation with yield parameters Similar results were also obtained when all the plants from untreated and treated plots were analysed together Further correlation study of per cent tunnel length in main stem, branches and whole plant also failed to show significant correlation with all the yield parameters. Use of frequency distribution data on yield parameters based on tunnel length in main stem, branches and whole plant also failed to reveal any definite influence on the yield parameters Use of frequency distribution of yield parameters based on

per cent tunnel length in main stem, branches and whole plant did not influence the yield parameters in a definite manner, Therefore, it was concluded that tunnel length or per cent tunnel length in no way affects the yield of soybean, Comparison of yield parameters between treated and untreated plots revealed that tunnel length in main stem and whole plant was higher in untreated plot as compared to treated plot Similar observations were also recorded with per cent tunnel length in main stem and whole plant However, all other yield parameters including seed weight per plant and weight per seed failed to show significant difference between treated and untreated plots, This finding clearly revealed that tunnel length failed to cause reduction of yield in soybean

(**Keywords** *Melanagtomyza sojae* / stem fly / stem borer / soybean)

Introduction

Soybean, *Glycine max* (L.) Merrill is an important legume for India as it contains high protein and oil, which will be able to meet the challenges of protein - energy malnutrition of the country. However, under field conditions this crop suffers from a variety of insect pests[1,2,3,4,5,6]. This is a major limiting factor in achieving higher productivity, Among the insects, *Melanagromyza sojae* (Zehntner) is well distributed in India[3,7], Australia, South Africa, Formosa, Saudi Arabia, Egypt[8], Indonesia[9], China[10] and Japan[11]. In Taiwan, it causes more damage in the autumn season than in the spring season[12]. In India, this insect is distributed in Delhi, Maharashtra, Madhya Pradesh, Karnataka, Uttar Pradesh, Himachal Pradesh[13] and hills as well as plains of Uttaranchal[3], It also causes extensive damage as borer to the soybean crop in the Orient, Africa and Oceania[14]. Although maggot causes damage to major portion of the pith region leaving xylem and phloem intact, the damage has little effect on supply of water and nutrients to the entire plant[8,15,16] while Chiang and Norris[17,18] reported that tunneling probably damages conducting tissues, especially xylem which is located close to the pith in soybean. This may result in reduced supply of water, nutrients from roots to the shoot and the damaged plants are significantly shorter. Singh and Singh[19,20] also reported the loss of plant vigour due to damage to conducting tissues. However, fully-grown plants when infested show no morphological deformities or symptom of insect attack. Hence, it is impossible to identify these damaged plants from uninfested plants[3]. Bhattacharya *et al.*[21] reported that tunnel length or damage due to *M. sojae* failed to show direct loss in the yield of soybean[22]. However, some reports are available which indicate that this insect causes loss in yield[20,23,24,25,26]. Therefore, an investigation was undertaken to find out the exact nature of economic losses caused by this insect in soybean. This investigation is of great value, as it would save money and environmental pollution if it were proved that no insectcidal application is needed to control this insect.

Materials and Methods

The experiment was carried out in the spring season. The field was divided into two plots. One plot was used as untreated plot while the other plot was used as treated plot. These paired plots (5 x 5 sq mt.) were maintained side by side with a gap 2.5 mt. In the treated plot Phorate (10G) was applied at the rate of 10 kg / ha in the furrows at the time of sowing and also on 30, 60 and 90 days after sowing to check the stem tunnelling by M. *sojae* Row to row distance in each plot was 45 cm. Soybean variety Bragg was sown in above plots following all agronomic practices. From each plot, 100 plants were randomly selected and uprooted during harvesting. These plants were individually kept in marked paper bags. Observations were recorded on tunnel length and per cent tunnel length in main stem, branches and whole plant. In addition, number of sub branches, number of sub-sub branches, total number of branches, height of the plant, total length of the branches, total length of the whole plant, number of pods per plant, weight of pods per plant, number of seeds per plant and weight of seeds per plant were also recorded. Weight per seed was also calculated from the above observation. For measuring the tunnel length in stem, each plant was split open with a knife. These plants were drawn on individual blank white paper sheets and data of each plant was recorded on the paper. Weight of the pods and seeds of individual plants were recorded with the help of digital electronic balance and seeds were counted manually by double-checking.

Correlation coefficient between tunnel length of stem, branches, whole plant and various yield components were calculated. Similarly, correlation coefficient was also carried out between per cent tunnel length of main stem, branches and whole plant and the foregoing yield components. These analyses were separately carried out for treated and untreated plots. In addition, correlation coefficient of the pooled data of treated and untreated plots were also carried out to obtain an overall status of yield characteristics[27]. Two-sample Z test were carried out for comparing equality of means between treated and untreated plots[28]. Data were analysed with the help of a computer.

Results and Discussion

1. Correlation between tunnel length and yield parameters

(a) Tunnel length in main stem: Correlation between tunnel length in the main stem and yield parameters in untreated plot revealed that the tunnel formation by the maggot of *M. sojae* in the main stem did not show any significant effect on yield parameters (Table 1). Similar result was also recorded when 100 plants from the treated plot were analysed (Table 2). Finally, the pooled analysis of untreated and treated plots together also showed similar result as mentioned above (Table 3).

(b) Tunnel length in branches: Correlation between tunnel length in branches and yield parameters were also carried out with 100 plants, each taken from treated and untreated plots. In both the plots tunnel formation caused by this insect in branches failed to show any detrimental effect in the yield parameters of soybean (Table 1 and 2). The pooled analysis of treated and untreated plots together also showed similar result as mentioned above (Table 3).

(c) Tunnel length in whole plant : Analysis of 200 plants together from treated and untreated plots, in correlation study, revealed that tunnel formation also failed to indicate significant reduction in yield parameters (Table 3). Earlier Bhattacharya *et al.*[21] also reported that tunnel length in the whole plant failed to show significant correlation with plant height, number of pods per plant, number of grains per plant, grain weight per plant and weight per grain.

II. Correlation between per cent tunnel length and yield parameters

(a) Per cent tunnel length in main stem: Correlation coefficient between per cent tunnel length in main stem and yield parameters indicated that the per cent tunnel length in main stem failed to show significant correlation with all the yield parameters of soybean plant in untreated and treated plots (Table 1 and 2). Similar trend was also observed when 200 plants of both treated and untreated plots were analysed (Table 3).

(b) Per cent tunnel length in branches: Computation of correlation between per cent tunnel length in branches and yield parameters did not indicate any significant relationship in 100 plants, each of untreated and treated plots (Table 1 and 2). Analysis of 200 plants also showed similar relationship (Table 3).

(c) Per cent tunnel length in whole plant: The correlation study between per cent tunnel length in whole plant and yield parameters failed to reveal any significant relationship between above parameters in untreated plot (Table 1). Similar relationship was also observed in treated plot (Table 2) while the combined analysis of untreated and treated plots showed a little variation. In this study, total length of the branches and total length of the plant showed significant decrease with the increase in per cent tunnel length in whole plant while yield parameters failed to show any significant relationship (Table 3).

III. Frequency distribution of yield parameters based on tunnel length

(a) Tunnel length in main stem : The study of frequency distribution of yield parameters in untreated and treated plots as well as a combined analysis revealed that

the mean yield parameters did not show a definite increase or decrease with the increase in tunnel length in main stem (Table 4)

(b) Tunnel length in branches: Frequency distribution based on tunnel length in branches was also investigated with 100 plants, each obtained from untreated and treated plots as well as for 200 plants pooled together. In this study all plants fell in a single class because tunnel length in branches was less as compared to main stem (Table 5).

(c) Tunnel length in whole plant: Frequency distribution of tunnel length in whole plant in relation to yield parameters in 100 plants, each from treated and untreated plots as well as a combined analysis of 200 plants from treated and untreated plots failed to indicate any definite trend in any of the yield characters of soybean (Table 6).

IV. Frequency distribution of yield parameters based on per cent tunnel length

(a) Per cent tunnel length in main stem: The frequency distribution in untreated and treated plots based on per cent tunnel length in main stem showed that not all the yield parameters differed significantly in different classes. Test with 200 plants from untreated and treated plots also revealed similar relationship (Table 7).

(b) Per cent tunnel length in branches and whole plant: The frequency distribution was also carried out based on per cent tunnel length in branches and whole plant. Table 8 and 9 revealed that the per cent tunnel length in branches or whole plant failed to influence the yield parameters in a definite manner.

Bhattacharjee[23] while working with soybean observed significant negative correlation between per cent stem length injury and yield as well as per cent stem length injury and height of the plant due to *Ophiomyia phaseoli*. The frequency distribution based on 5 per cent stem length injury (except first two observations in which class interval was 2.5) showed a decreasing average yield per plant and average height per plant with increase in per cent stem length injury. However, Kundu and Mehra[26] working in the same laboratory reported that stem fly attacking soybean in the field was M. *sojae* and not O. *phaseoli* as described earlier by Bhattacharjee[23]. They found that all the yield components showed significant negative correlation with tunnelling per centage. Frequency distribution with 10 per cent tunnel length as class - interval showed that mean in different classes of tunnelling per centage was different for grain weight, pod number, plant height, pod weight per plant and grain number per plant. They also indicated that major yield component which was affected due to attack of this insect was number of pods per plant, which indirectly affected the grain weight per plant. Similarly, Singh and Singh[20] stated that stem tunnel length caused by M. *sojae* reduced the grain yield.

Table 1– Correlation coefficient between tunnel length and yield parameters as well as among yield parameters of soybean variety Bragg for 100 plant samples from untreated plot

Parameters	Height of the plant	Total length of all branches	Total length of plant	No of sub branches	No of sub-sub branches	Total no of branches	No of pods per plant	No of seeds per plant	Seed weight per plant	Weight per seed
Per cent T.L. in main stem	-0 050	0 051	0.016	0.069	0.098	0.095	0.056	0 053	0 101	0 124
Per cent T L. in branches	-0.008	0 061	0 012	0.107	-0 017	0.082	0 123	0 159	0.156	0.054
Per cent T L in whole plant	-0.1 04	-0.085	-0 110	-0.042	0.037	-0.021	-0 013	-0 011	0 052	0 131
T L in main stem	0 066	0.110	0 108	0 104	0 088	0.121	0.097	0 096	0.124	0.106
T.L in branches	-0 064	0 082	0.028	0 106	-0 017	0.082	0 129	0 181	0 150	0 024
T.L in whole plant	0 007	0.015	0 012	-0 073	0 050	-0 042	-0 014	0 018	0 010	0 105
Height of the plant				0 188	-0.046	0 136	0 422**	0 385**	0 226*	-0 117
Total length of branches				0.729**	0 229*	0 694**	0 614**	0 604**	0 430**	-0 103
Total length of plant				0 630**	0 156	0 584**	0 638**	0 614**	0.419**	-0 124

Table 1 Contd

Table 1 Contd

No of sub branches	0 397**	0 387**	0 244*	-0 109
No of sub-sub branches	0 194	0 148	0 147	0 024
Total no of branches	0 405**	0 379**	0 259**	-0 081
No of pods per plant		0 888**	0 746**	0 013
No. .)f seeds per plant			0 802**	-0 042
Seed weight per plant				0 536**

T L = Tunnel length *Significant at 5 per cent level of significance **Significant at 1 per cent level of significance

Table 2– Correlation coefficient between tunnel length and yield parameters as well as among yield parameters of soybean variety Bragg for 100 plant samples from treated plot

Parameters	Height of the plant	Total length of all branches	Total length of plant	No of sub branches	No of sub-sub branches	Total no of branches	No of pods per plant	No of seeds per plant	Seed weight per plant	Weight per seed
Per cent T.L in main stem	-0.080	-0 084	-0 109	0.026	0.000	-0 043	-0 120	-0 094	0 022	0 109
Per cent T L. in branches	0.000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
Per cent T L in whole plant	-0.076	-0.101	-0.122	-0.008	0 000	-0 027	-0.127	-0 103	0 024	0 128
T.L in main stem	-0 035	-0 078	-0 090	-0 025	0 000	-0 042	-0 132	-0 109	0 000	0 088
T.L in branches	0 000	0.000	0 000	0 000	0 000	0 000	0 000	0 000	0 000	0 000
T L in whole plant	-0 040	-0 067	-0.081	-0 011	0 000	-0 029	-0 125	-0 104	-0 195	0 101
Height of the plant				0 042	0 000	0 100	-0 003	-0 101	-0.195	-0 179
Total length of branches				0 616**	0 000	0 549**	0 361 **	0 269**	0 018	-0 306**
Total length of plant				0 549**	0 000	0 512**	0 331**	0 212*	-0 025	-0 314**

Table 2 Contd

Table 2 Contd

No. of sub branches	0 384**	0 289**	0 080	-0 270**
No of sub-sub branches	0 000	0 000	0 000	0 000
Total no. of branches	0 282**	0 247*	-0 035	-0 375**
No. of pods per plant		0.865**	0 701 **	-0 078
No of seeds per plant			0 778**	-0 132
Seed weight per plant				0 493**

T.L. = Tunnel length

* Significant at 5 per cent level of significance

** Significant at 1 per cent level of significance

Table 3– Correlation coefficient between tunnel length and various yield parameters of 200 plant samples from treated and untreated plots

Parameters	Height of the plant	Total length of all branches	Total length of plant	No of sub branches	No. of sub-sub branches	Total no. of branches	No of pods per plant	No of seeds per plant	Seed weight per plant	Weight per seed
Per cent T L in main stem	-0 100	-0 127	-0 153	-0 078	0 115	-0 053	0 016	-0 011	0 035	0 069
Per cent T L in branches	-0 057	0 005	-0.022	0.052	-0 009	-0.037	- 0 091	0,110	0 108	0 033
Per cent T L in whole plant	-0 1 26	-0.183**	-0 208**	-0 133	0.069	-0 111	-0 022	-0 047	0 008	0 077
T.L in main stem	-0.029	-0.099	-0 105	-0 056	0 108	-0 037	0 037	0 011	0 044	0 056
T.L. in branches	-0.054	0 016	-0.012	0 051	-0 009	0 037	0 096	0 126	0 104	0 012
T L in whole plant	-0 034	-0.093	-0 101	-0 045	0 104	-0.028	0 048	0 025	0 056	0 058
Height of the plant				0 128	-0 038	0 131	0.204**	0 142*	0 018	-0 146*
Total length of branches				0 654**	0 105	0 605**	0 435**	0 388**	0 186**	-0 212**

Table 3 Contd

Table 3 Contd.

Total length of plant	0 594**	0 073	0 552**	0 441**	0 379**	0 173*	-0 220**
No. of sub branches				0 381**	0 341**	0 172*	-0.180*
No. of sub-sub branches				0 142*	0.105	0.104	0 014
Total no. of branches				0 339**	0 317**	0 125	-0 221**
No. of pods per plant					0 877**	0 724**	-0 033
No. of seeds per plant						0 791**	-0 085
Seed weight per plant							0 514**

T.L = Tunnel length, *Significant at 5 per cent level of significance **Significant at 1 per cent level of significance

Table 4 – Frequency distribution of various yield parameters of soybean based on tunnel length in the main stem caused by *M sojae* in plants from untreated and treated plots.

Class range	Tunnel length (cm)	Freq-uency	Plant height (cm)	Length of all branches (cm)	Total plant length (cm)	No of sub branches /plant	No. of sub-sub branches/ plant	Total no of branches/ plant	Pod no / plant	Seed no / plant	Seed Wt / plant (g)	Weight / seed (g)
100 Plants from untreated plot												
0-5	2.5	79	35 9	9.5	45 4	3.2	0 0	3 2	25 7	43 0	4 2	0 099
5-10	7.1	17	35 8	9 5	45 4	3 5	0.2	3 8	26 7	46 0	5 0	0 112
1 0-1 5	12 1	3	40 0	19 5	59 1	4 3	0 0	4 3	35 6	59 0	6 0	0 104
15-20	17.5	1	35 5	3 5	39 0	2 0	0 0	2 0	25 0	33 0	2.6	0 080
100 Plants from treated plot												
0-5	0 3	99	36.7	13 5	50 6	3 7	0 0	3 8	25 9	44 8	4 5	0.102
5-10	7.0	1	34 0	8 5	42 5	4 0	0 0	4 0	25 0	48 0	5 2	0 109
200 Plants from untreated and treated plots (pooled analysis)												
0-5	1 3	178	36.3	11.7	48 3	3 5	0 0	3 5	25 8	44 0	4 4	0 101
5-10	7.1	18	35 7	9 5	45 2	3 5	0 2	3 8	26 6	46 1	5 0	0 112
1 0-1 5	12.1	3	40 0	19 5	59.1	4 3	0 0	4 3	35 6	59 0	6 0	0 104
15-20	17 5	1	35 5	3 5	39 0	2 0	0 0	2 0	25 0	33 0	2 6	0.080

Table 5– Frequency distribution of various yield parameters of soybean based on tunnel length in the branches caused by *M soyae* in plants from untreated and treated plots

Class range	Tunnel length (cm)	Freq-uency	Plant height (cm)	Length of all branches (cm)	Total plant length (cm)	No of sub branches / plant	No of sub-sub branches/ plant	Total no of branches/ plant	Pod no./ plant	Seed no / Plant	Seed Wt / plant (g)	Weight /seed (g)
100 Plants from untreated plot												
0-5	0 0	100	36 0	9 8	45 8	3 3	0 0	3 3	26 1	43 9	4 4	0 101
100 Plants from treated plot												
0-5	0 0	100	36 7	13.5	50 5	3 7	0 0	3 8	25 9	44 8	4 5	0 103
200 Plants from untreated and treated plots (pooled analysis)												
0-5	0 0	200	36 3	11.6	48 1	3 5	0 0	3 6	26 0	44 4	4 5	0 102

Table 6– Frequency distribution of various yield parameters of soybean based on absolute tunnel length in the whole plant caused by *M sojae* in plants from untreated and treated plots

Class range	Tunnel length (cm)	Freq-uency	Plant height (cm)	Length of all branches (cm)	Total plant length (cm)	No of sub branches / plant	No of sub-sub branches/ plant	Total no of branches/ plant	Pod no / plant	Seed no / Plant	Seed Wt/ plant (g)	Weight/ seed (g)
100 Plants from untreated plot												
0-5	2 5	79	35.9	9.5	45 4	3 2	0 0	3 2	25.7	43 0	4 2	0 099
5-10	7.2	17	35 8	9 5	45 4	3.5	0 2	3 8	26 7	46 0	5 0	0 112
1 0-15	13 1	3	40 0	19 5	59 1	4 3	0 0	4 3	35 6	59 0	6.0	0 104
15-20	17.5	1	35 5	3.5	39 0	2.8	0 0	2 0	25.0	33 0	2 6	0 080
100 Plants from treated plot												
0-5	0 3	99	36 7	13.5	50 6	3 7	0 0	3 8	25 9	44 8	4 5	0 102
5-10	7.0	1	34 0	8 5	42.5	4 0	0 0	4 0	25 0	48 0	5 2	0 109
200 Plants from untreated and treated plots (pooled analysis)												
0-5	1 3	178	36.3	11 7	48.3	3 5	0 0	3 5	25 8	44 0	4 4	0 101
5-10	7 2	18	35.7	9 5	45 2	3.5	0 2	3.8	26 6	46 1	5 0	0 112
1 0-15	13.1	3	40.0	19 5	59 1	4 3	0 0	4 3	35 6	59 0	6 0	0 104
15-20	17 5	1	35 5	3 5	39 0	2.0	0 0	2.0	25 0	33 0	2 6	0 080

Table 7– Frequency distribution of various yield parameters of soybean based on per cent tunnel length in the main stem caused by M *sojae* in plants from untreated and treated plots

Class range	Tunnel length (%)	Freq-uency	Plant height (cm)	Length of all branches (cm)	Total plant length (cm)	No of sub branches /plant	No of sub-sub branches/ plant	Total no of branches/ plant	Pod no / plant	Seed no /Plant	Seed Wt / plant (g)	Weight / seed (g)
					100 Plants from untreated plot							
0-5	0.3	28	36 9	9 1	46 0	3 1	0.0	3 1	25 9	42 7	4 3	0 101
5-10	6 9	28	36.0	11 1	47 1	3 6	0 0	3 6	27 6	46 8	4 4	0 096
10-15	11.9	19	34 8	8 3	43 2	3 1	0 0	3 1	23 3	38 3	3 6	0 096
15-20	19 6	16	34 8	9 0	43 9	3 2	0 3	3 5	26 8	46 8	5 1	0 110
20-25	21 2	3	35.3	9 6	45 0'	4 0	0 0	4 0	20 6	34 6	4 2	0 126
25-30	28 3	4	39 6	15 6	55 2	4 0	0 0	4 0	31 0	50 5	6 0	0 119
30-35	32 8	1	36 5	15 5	51 0	4 0	0 0	4 0	37 0	68 0	5.4	0 080
45-50	49.2	1	35 5	3 5	39 0	2 0	0 0	2 0	25 0	33 0	2 6	0 080
					100 Plants from treated plot							
0-5	0 1	0 88	36.6	13 7	50 8	3 7	0 0	3 8	26 3	45 4	4 5	0 102
5-10	7 3	10	37 1	12 6	49 7	3 4	0.0	3 4	22 4	39 4	4 2	0 104
10-15	10 6	1	37 5	5 5	43.0	4 0	0 0	4 0	27 0	42 0	5 9	0 142
15-20	20.5	1	34 0	8 5	42.5	4.0	O.C'	4 0	25 0	48 0	5 2	0 109

Table 7 Contd

Table 7 Contd.

200 Plants from untreated and treated plots (pooled analysis)												
0-5	0.1	116	36 7	12 6	49 6	3.6	0 0	3 6	26 2	44 8	4 5	0 102
5-10	7.0	38	36 3	11.5	47.8	3.5	0 0	3 5	26 2	44 8	4 4	0 098
10-15	11.8	20	35.0	8 2	43 2	3 2	0 0	3 2	23 5	38 5	3 7	0 098
15-20	19 6	16	34.8	9 0	43 9	3 2	0.3	3 5	26 8	46 8	5 1	0 110
20-25	21 0	4	35 0	9 3	44 3	4.0	0 0	4 0	21 7	38 0	4 4	0 122
25-30	28.3	4	39 6	15 6	55 2	4 0	0 0	4 0	31 0	50 5	6 0	0 119
30-35	32 8	1	36 5	15 5	51.0	4 0	0 0	4 0	37 0	68.0	5 4	0 080
45-50	49.2	1	35 5	3 5	39.0	2 0	0.0	2 0	25 0	33 0	2 6	0 080

Table 8– Frequency distribution of various yield parameters of soybean based on per cent tunnel length in the branches caused by *M sojae* in plants from untreated and treated plots

Class range	Tunnel length (%)	Freq-uency	Plant height (cm)	Length of all branches (cm)	Total plant length (cm)	No of sub branches / plant	No. of sub-sub branches/ plant	Total no of branches/ plant	Pod no / plant	Seed no / plant	Seed Wt / plant (g)	Weight/ seed (g)
100 Plants from untreated plot												
0-5	0 0	97	36 0	9 7	45 7	3 3	0 0	3 3	26 0	43 6	4 3	0 101
1 0-15	11.5	1	32.0	13 0	45 0	4 0	0 0	4.0	23 0	47.0	5 9	0 126
15-20	19.3	1	36.5	15.5	51 0	4 0	0 0	4 0	37 0	68 0	5 4	0.080
20-25	23.5	1	35 0	8 5	43.5	4 0	0.0	32 0	50 0	50 0	5 8	0 118
100 Plants from treated plot												
0-5	0 0	100	36 7	13.5	50.5	3 7	0 0	3 8	25 9	44 8	4 5	0 103
200 Plants from untreated and treated plots (pooled analysis)												
0-5	0.0	197	36.3	11.6	48.2	3.5	0.0	3.5	26.0	44 2	4.4	0 102
10-1 5	11.5	1	32.0	13 0	45 0	4 0	0 0	4 0	23 0	47 0	5 9	0.126
15-20	19 3	1	36 5	15 5	51 0	4 0	0 0	4 0	37.0	68.0	5.4	0 080
20-25	23.5	1	35.0	8.5	43 5	4 0	0 0	4 0	32 0	50 0	5 8	0 118

Table 9– Frequency distribution of various yield parameters of soybean based on per cent tunnel length in the whole plant caused by *M sojae* in plants from untreated and treated plots

Class range	Tunnel length (%)	Freq-uency	Plant height (cm)	Length of all branches (cm)	Total plant length (cm)	No of sub branches / plant	No of sub-sub branches/ plant	Total no of branches/ plant	Pod no / plant	Seed no / Plant	Seed Wt / plant	Weight/ seed (g)
					100 Plants from untreated plot							
0-5	1 3	35	36 6	10.1	46.7	3.3	0 0	3 3	26.3	43.6	4 3	0 099
5-10	7.1	30	35 9	10 2	46 1	3.3	0 0	3 3	26 9	45 8	4 3	0 096
10-15	12 1	20	34.8	8 8	43.7	3 3	0 2	3 5	24 3	40 6	4 3	0 106
15-20	16 7	10	35 5	9 2	44 8	3 5	0 0	3 5	25 8	43 7	4 6	0 112
20-25	22 1	3	38 5	10 5	49 0	3 3	0 0	3 3	28 0	47 0	5 8	0 123
25-30	29.4	1	36 5	15 5	51 0	4 0	0 0	4 0	37 0	68 0	5 4	0 080
40-45	44.8	1	35 5	3 5	39 0	2 0	0 0	2 0	25 0	33 0	2 6	0 080
					100 Plants from treated plot							
0-5	0.2	92	36 7	13 7	50 8	3.7	0 0	3 8	26 1	45 1	4 5	0 101
5-10	6 5	7	36 1	11 0	47 2	3 5	0 0	3 5	23 5	40 2	4 7	0 117
15-20	16 4	1	34 0	8 5	42.5	4 0	0 0	4 0	25 0	48 0	5 2	0 109

Table 9 Contd

200 Plants from untreated and treated plots (pooled analysis)												
0-5	0.5	127	36 7	12 7	49 7	3 6	0 0	3 7	26 2	44 7	4 5	0 101
5-10	7.0	37	35 9	10 3	46 3	3 3	0 0	3 3	26 2	44.7	4 4	0 100
10-15	12 1	20	34 8	8 8	43.7	3 3	0 2	3 5	24 3	40 6	4 3	0 106
15-20	16 6	11	35 4	9.1	44 5	3 5	0 0	3 5	25 7	44 0	4 7	0 111
20-25	22 1	3	38 5	10 5	49 0	3 3	0 0	3 3	28 0	47 0	5 8	0 123
25-30	29 4	1	36 5	15 5	51 0	4 0	0 0	4 0	37 0	68 0	5 4	0 080
40-45	44.8	1	35 5	3 5	39 0	2 0	0 0	2 0	25 0	33 0	2 6	0 080

Table 10– Effect of tunnel length caused by *M sojae* and other plant characters on the yield of soybean

Characters	Treated	Untreated	Calculated Z value
Tunnel length in main stem (cm)	2 8	4 7	-5 840**
Per cent tunnel length in main stem	8 0	13 2	-6 051**
Tunnel length in branches (cm)	0 0	2 3	–
Per cent tunnel length in branches	0 0	19 4	–
Tunnel length in whole plant (cm)	2 9	4 8	-5 735**
Per cent tunnel length in whole plant	6 2	10 7	-6 038**
Plant height (cm)	36 7	36 0	1 429^{NS}
Length of all branches (cm)	13.5	9 8	3 194**
Total length of plant (cm)	50 5	45 8	3 381**
Number of sub branches	3 7	3 3	1 542^{NS}
Number of sub-sub branches	0.0	0 0	-
Total number of branches	3 8	3 3	1 374^{NS}
Number of pods per plant	25 9	26 1	-0 222^{NS}
Pod weight per plant (g)	-	-	-
Number of seeds per plant	44 8	43 9	0 532^{NS}
Seed weight per plant (g)	4 5	4 4	0 646^{NS}
Weight per seed (g)	0.1	0.1	0 00^{NS}

*Significant at 5 per cent level of significance

**Significant at 1 per cent level of significance

At Delhi[23,26] and Sehore, Madhya Pradesh[20] maximum tunnelling per centage ranged from 90 to 100 per cent. Although 100 per cent infestation of soybean plants by M. *sojae* may occur in the field, but at the same time 90 to 100 per cent tunnel formation, which means tunneling by M. *sojae* larvae continuously from tip of the plant to the ground level of the plant, appears to be over emphasis on the tunnelling behaviour by this insect. Most interestingly, 90 to 100 per cent stem tunnelling was not reported from Pantnagar, Parbhani, Indore, Pune, Ranchi and Banglore. Even at Delhi and Sehore data collected in last 10 years showed per centage stem tunnelling ranging from 30 to 80 and 28 to 86, respectively, except in 1987 at Delhi and Sehore[22]. Therefore, it appears that observations recorded by Bhattacharjee[23], Kundu and Mehra[26] and Singh and Singh[20] had some error on per centage stem tunnelling which finally affected the yield components. They also showed with the frequency distribution data, a clear trend of decreasing order of yield components with increasing per centage tunnel length, which was not recorded in this detailed investigation. Talekar and Chen[12] without analyzing the overall observations of Bhattacharjee[23], supported these results in graphic manner and showed decrease in yield with increase in per cent stem length injury. It is also interesting to note that Bhattacharjee[23] indicated his work on O. *phaseoli* (= *Melanagromyza sojae)* but Talekar and Chen[12] interpreted the same data using *M sojae.* The feeding behaviour of these two insects reveal that *M. sojae* is a pith feeder while O. *phaseoli* feeds through the cortical layer beneath the epidermis of stem[8]. Therefore, it is obvious that Bhattacharjee[23] did not properly identify the insect on which he was working. Furthermore, it is emphasised that these workers computed per cent tunnel length based on main stem only as if tunnels made by the maggot of M. *sojae* in branches and sub branches have no effect on the yield components of soybean. This has also resulted in misrepresentation of data and yield character of soybean. Present study amply provide evidences which indicate that tunnel formation by this insect in no way affect the grain yield of this crop. Therefore, application of systemic insecticide is not needed to control this insect.

V. Comparison of yield parameters between treated and untreated plots by Z test

The comparison of mean values of tunnel length; per cent tunnel length and yield parameters was carried out between treated and untreated plots. Table 10 indicates that the tunnel length in the main stem and whole plant was significantly higher in untreated plot as compared to treated plot. Similar trend was also observed when per cent tunnel length in the main stem and whole plant was considered. In this season, no significant difference in the height of the plant was observed in treated and untreated plots while the length of the branches and the total length of the plant were

significantly higher in treated plot as compared to untreated plot. Most interestingly all other yield parameters including seed weight per plant and weight per seed did not show significant difference between treated and untreated plots. Talekar[24] while studying the affect of infestation of *M. sojae* on soybean at Taiwan observed that 1000 seed weight failed to show significant difference between protected and unprotected plots. However, a significant increase in yield (kg/plot) was recorded in protected plot. It is difficult to understand such a difference. In fact his emphasis on the reduction in leaf area caused due to M. *sojae* should imply on the difference in 1000 seed weight also.

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Shelf life studies of *Pleurotus florida* Sing.

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Abstract

The fresh mushrooms, after harvest continue their physiological processes and enter into a rapid phase of senescence They are very delicate, contain 90% water, rich in degradatory enzymes, lack a protective covering of suberin or cuticle, unlike fresh fruits and vegetables At room temperature they respire very fast, tend to lose water content, turn brown and develop off-flavor coupled with change in texture. They cannot be held in acceptable condition for more than 24 hours at room temperature which directly affect their economic value. Therefore in present communication the studies on extended shelf life of fresh produce of *Pleurotus florida* has been evaluated at two different temperature ranges (8-10 °C & 28- 30 °C) by storing in three different containers that is, open plastic tray, perforated polythene packets and perforated brown paper packets Out of these the perforated polythene packets was found to be the most efficient and sustained the maximum shelf life of 7 days at the temperature of 8-10°C.

(Keywords: shelf life / mushroom / polythene packets / temperature)

Introduction

Diversified agro-climatic conditions in India offer vast potential for growing different types of mushrooms. In India three, viz, white button mushroom *(Agaricus bisporus)*, Oyster mushroom *(Pleurotus* spp.) and Paddy straw mushroom *(Volvariella* spp.) are grown commercially. Out of these, various species of *Pleurotus* predominates and are cultivated commercially throughout the world. They have been recognized as the alternate source of good quality protein and are producing the highest quality protein per unit area and time from the worthless agro-wastes[5-7]. Despite their increased production, they are always subjected to the danger of spoilage due to their high moisture content, delicate nature, and various senescence activities. Thus, due to short shelf life of the produce, they remain acceptable for few hours only at the high ambient temperature of the tropics and sub-tropics. This, in turn, causes serious economic losses by deteriorating their qualities of importance like reduction in weight, changes in colour, texture, odour, taste and other morphological

characteristics[3] The shelf life is one of the important characteristic of any food substance by virtue of which it may be able to keep itself fresh and its quality remains the same for sometimes under certain temperature ranges. Temperature and the kind of storing containers are the important limiting factors affecting the shelf life of the mushrooms. Therefore, to find out prolonged storage life of fresh mushroom, studies on shelf life of *Pleurotus floridan,* important edible mushroom of Madhya Pradesh, were made at two temperature ranges.

Materials and Methods

Shelf life studies of freshly cultivated *Pleurotus florida* were made just after harvesting good and healthy fruitings as per method followed by Nicholas[4]. These studies were conducted in open plastic containers, wrapping in perforated brown paper packets and 100 gauge perforated polythene packets. The experiment was conducted at 8- 10°C and 28-32°C (room temperature) temperature. Mushroom samples weighing 100gm were used for this experiment, in replicates of five. Weight loss, colour change, odour change and other morphological changes were recorded every 24 hours until the samples became inedible or spoiled.

Results and Discussion

Mushroom is a highly perishable crop and its proper storage and processing is one of the important aspect in post harvest technology of mushroom cultivation. Therefore, looking to its importance, the shelf life studies of the important edible mushroom, *Pleurotus florida,* was performed to find out the optimum temperature and suitable container for storing the harvested mushroom for longer duration. The result obtained revealed that the mushroom samples stored in perforated polythene packets at the temperature ranges of 8-10 and 28-30 °C supported maximum shelf life of 7 days and 5 days respectively. The perforated brown paper packets ranked next and supported the maximum shelf life of 3 and 4 days at the temperature range of 8-10 and 28-30 °C respectively. The mushroom samples stored in open container at both the temperature ranges could not sustain the shelf life of more than 2 days. Overall it was observed that the lower temperature range of 8-10 °C and perforated polythene packets were proved to be the best in sustaining the maximum shelf life. Nicholas[4] also obtained similar results and reported that the shelf life of mushrooms can often be extended by storing at lower temperatures. The results pertaining to detailed visual observations are shown in Table 1 and 2.

Table 1– Shelf life of *Pleurotus florida* at 8-10°C

S No	Visual observation	Storage	Storage period (Days)						
			2	3	4	5	6	7	8
1.	Weight loss	OC	–	43	58	66			
		BP	–	–	10	28	62		
		PP		–	–	12	20	44	60
2.	Colour Change	OC		–	Sporophore start yellowing	Sporophore become complete yellow & blackened			
		BP	–	–	–	Sporophore yellowish	Sporophore yellowish brown		
		PP	–	–	–	–	Slight yellowish	Yellow	yellowish blackening
3.	Odour change	OC	–	–	Not agreeable	Foul			
		BP	–	–	–	Not agreeable	Foul		
		PP	–	–	–	–	–	Not agreeable	Foul

Table 1 Contd .

Table 1 Contd...

4	Other morphological changes	OC	–	–	Sporophore start shrinking, drying & softening	Sporophore shrinked, dried & softened			
		BP	–	–	–	Sporophore start drying, softening & shrinking	Sporophore shrinked, dried & softened		
		PP	–	–	–	Very slight sofetening	Sporophore shrinked, dried & softened		
5	Edibility	OC	–	+	*	Spoiled			
		BP	+	+	+	*	Spoiled		
		PP	+	+	+	+	+	*	Spoiled

OC : Open Container
PP Perforated Polythene Packet
\+ . Acceptable
BP Brown Perforated Paper Packet
– No change
* Not Acceptable

Table 2– Shelf life of *Pleurotus florida* at 28-30°C

S. No.	Visual observation	Storage	Storage period (Days)						
			2	3	4	5	6	7	8
1.	Weight loss	OC	–	22	48	63			
		BP	–	10	36	67			
		PP	–	–	08	14	46	66	
2	Colour Change	OC	–	Sporophore start yellowing	Sporophore yellow, blackening	Sporophore blackened			
		BP	–	–	Sporophore start yellowish	Sporophore yellow, blackened			
		PP	–	–	Sporophore slight yellowing	Sporophore start yellowing	Sporophore yellow with blackening	Sporophore blackened	
3.	Odour change	OC	–	–	Not agreeable	Foul			
		BP	–	–	Not agreeable	Foul			
		PP	–	–	–	–	Not agreeable	Foul	

Table 2 Contd

Table 2 Contd...

4.	Other morphological changes	OC	–	Sporophore start shrinking	Sporophore start shrinking & drying	Sporophore shrinked & dried		
		BP	–	–	Sporophore start shrinking, drying & softening	Sporophore shrinked & dried		
		PP	–	–	–	–	Sporophore start shrinking, softening & drying	Sporophore shrinked, softened & dried
5.	Edibility	OC	+	*	*	Spoiled		
		BP	+	+	*	Spoiled		
		PP	+	+	+	*	*	Spoiled

OC : Open Container
BP : Brown Perforated Paper Packet
PP : Perforated Polythene Packet
– : No change
+ : Acceptable
* : Not Acceptable

The use of suitable packaging materials like polythene and polypropylene extend the shelf life by retarding water loss and by conserving the freshness of mushrooms. Low temperature storage results in effective short term storage by retarding rapid phase of senescence by various post harvest metabolic activities of mushroom tissues, deteriorative chemical reactions and moisture loss. Besides, the perforated polythene bags are permeable to oxygen and carbon dioxide. Respiring tissues consume oxygen and produce carbon dioxide, thus, depleting the oxygen and increasing the carbon dioxide in the air around the tissue contained in wrapped polythene packets. The concentration of the gases reach an equilibrium which is a function of the rate of respiration of the tissue and the permeability of the polythene packets. In fact this creates a controlled atmosphere, capable of keeping the mushroom fresh[1]. Gormley and MacCanna[2] found that the shelf life of mushrooms increased by over wrapping them with Poly Vinyl Chloride (PVC) films.

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Phosphate solubilizing activity of endophytic bacteria isolated from sugarcane plant

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Abstract

Twelve endophytic bacteria isolated from sugarcane were screened for phosphate solubilizing activity (PSA) Nine of them showed PSA which were further evaluated for their interaction with sugarcane plant Most of them significantly affected the plant germination, physiological parameters and total phosphorus content of plant

(Keywords endophytes/phosphate solubilizing activity/rock phosphate/insoluble phosphate)

Introduction

Phosphorus is one of the major plant nutrients limiting growth and involved in many essential processes including cell division, photosynthesis, breakdown of sugars, energy transfer and nutrient transfer within plants[1]. The role of P in expression and maintenance of genetic material is well established [2] Indian soils contain low to medium amount of available P. Approximately 49.3% district, and union territories are low in available P and other 48.8% have medium level of P[3]. This highlights the need of phosphatic fertilizer application for proper crop growth and better yields in nearly 98% of Indian districts. Much of the P applied to soil through inorganic fertilizers is rapidly fixed in to insoluble form as Ca, Fe, and Ai salt and rendered essentially available to plant. However, fixed P is converted into plant available forms by some saprophytic soil micro-organism as well as by plants roots, and mycorhizal fungi, which bring about changes in pH of the soil environment and produce chelating substances[4]. Solubilization of rock phosphate (RP) by P-solubilizing micro-organisms

(PSB) has also been reported [3, 6-8]. A large number of PSB have been isolated from the rhizosphere of several crops. However, much information is not available on PSB from endophytes of sugarcane plants

During the present investigation PSA of twelve sugarcane endophytic bacteria were studied and further tested on sugarcane plants to examine the plant -bacterium interaction.

Materials and Methods

Bacterial cultures used in the present investigation were strain no. 100, 101, 113, 120, 121, 127, 128, 29-0, 150 and 151 of sugarcane endophytic bacteria Bacterial cultures were maintained on LGI[9] medium containing (gL^{-1}) K_2HPO_4; 0 2, $MgSO_4.7H_20$; 0.2, $CaCl_2.2H_2O$, 0.02, $Na_2MoO_4.2H_2O$; 0.002, $FeCl_3$ $6H_2O$; 0.01, Bromothymol blue 5ml (0.2 M in 0.2% KOH) sucrose 10.0, Agar 1 8% and 1000 ml of double distilled water.

Bacterial isolates were screened for PSA on the medium containing unavailable form of P in Pikovskaya's[10] and modified HAM medium[11]. Tri-calcium phosphate (TCP) precipitates of K_2HPO_4 in Pikovskaya's and HAM medium[11] respectively, were used as insoluble sources of P. For precipitation, after autoclaving the media, separately autoclaved $CaCl_2$ (10%) and K_2HpO_4 (10%) was added at the rate of 3ml 100^{-1} and 2ml100^{-1}, respectively The solid agar was added in both medium Plates of above medium were spot inoculated with the individual test cultures and incubated at 35°C up to 10 days. Formation of clear zone around the colonies was considered as positive. The ability of bacteria to solubilize insoluble P was described by solubilization index, which is the ratio of total diameter (Colony + Holozone) and diameter of colony.

The PSA of bacterial isolates was also measured in liquid medium of HAM and Pikovskaya's. Conical flasks containing 100 ml of liquid medium were inoculated with a loopful of 48 hours old pure culture, and were incubated for 10 days under shake culture condition. Concentration of P was estimated spectrophotometricaly in the culture supernatants by the method of Gaur (1990)[3].

The response of PSB on plant growth and assimilation of P was also studied. Bud chips of sugarcane COLK 8102 variety were inoculated with the test cultures and the inoculated buds were planted on pots containing sterilized soil amended with RP. The germination percentage was recorded after 15 days and different physiological parameters like shoot length, fresh weight, number of leaves and dry weight were recorded at one month stage.

The total phosphorus concentration in dried plant samples was determined spectrophotometricaly.

Results and Discussion

The PSA of bacterial isolates is shown in Fig. 1. The zone of clearance around the colonies indicates positive activity (i.e. A-E, H, K, L) whereas the colonies without any zone of clearance are negative (i.e. G I, J,).

The P-solubilization index (PSI) of the test isolates in HAM and Pikovskaya 's medium ranges from 1.0 to 2.22. (Table-1) The highest PSI in HAM was shown by isolates no. 121 (2.22) followed by 120 (2.10) and 111, 150 (1.92), whereas in Pikovskaya's medium the highest PSI was shown by isolates no101 (1.24) followed by 111 (1.42) and 151 (1.35). Solubilization of P by micro-organism was mainly due to the production of organic acid and chelating substances. Our isolates produce acidity while utilizing sucrose and pH was reduced to 2.5-3.0. The PSA by isolates were also determined in liquid medium (Table-2). It is observed that in Pikovskaya's broth, formation of soluble P was more as compared to HAM broth. Maximum P-solubilization was recorded from the isolate no. 107 followed by 111, 101, 121, 150 and 151. Along with the growth of these organisms the decline in pH was also recorded. In HAM medium K_2HPO_4 precipitated with $CaCl_2$ was used as insoluble source of P, whereas in Pikovskaya's medium (TCP) tri-calcium phosphate was used as insoluble source of P. The strain we studied was able to solubilize phosphorus utilizing K_2HPO_4 precipitated with calcium chloride. The variation in P solubilization was due to source of P supplied in the medium as reported earlier.[5-6,11]

Table 1– P-Solubilization Index of sugarcane endophytic bacteria

Sl No	Bacterial Isolates	P-Solubilization Index PSI	
		In HAM Medium	In Pikovskaya's Medium
1	100	1 70	1 28
2	101	1 68	1 46
3	107	1.60	1 26
4	111	1 74	1.42

Table 1 Contd.

Table 1 Contd

5	113	1 92	1 28
6	120	2 10	1 31
7	121	2 22	1.12
8	127	1.0	1 0
9	128	1.0	1 0
10	29-0	1 0	1.0
11	150	1 9	1.2
12	151	1.46	1 2

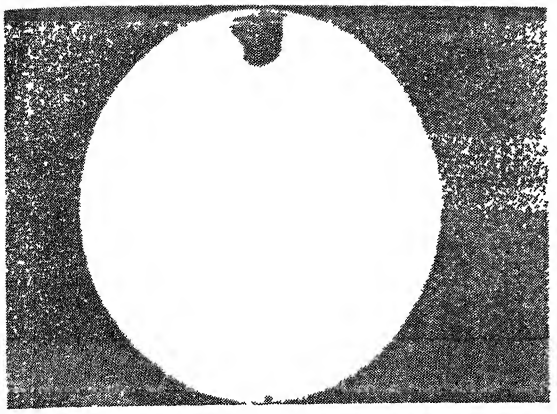
$$PSI = \frac{Diameter\ of\ Holozone + Colony}{Diameter\ of\ Colony}$$

1 0 is considered as negative P-solubilizing activity, Incubation period 10 days

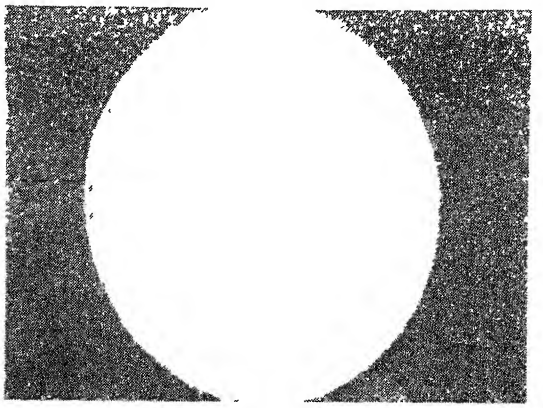
Table 2– P-Solubilization in HAM and Pikovskaya's Broth

Sl No.	Bacterial Strains	P-Solubilization in liquid	
		In HAM Broth	In Pikovskaya's Broth
1	100	93.00	130 1*
2	101	17.09	180.0***
3	107	104 3	337 0**
4	III	139 6	281 7*
5	113	93 8	222.0*
6	120	124 8	212 9*
7	121	74 1	256 4**
8	150	133.1	240 6*
9	151	50 7	246 6***

Incubation period 10 days, *P<0 05, **P<0 01, ***P<0 001



(A)



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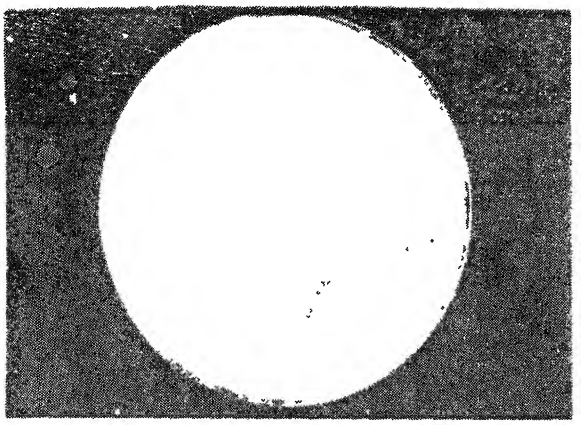

(C)

Fig 1– Zone of P-solubilization around the colony
(A) Isolates No A 101,B 100, C Ill, D 107
(B) Isolates No E 120, F113, G127, H 121
(C) Isolates No I 29-0, J 128, K 151, M 150

The effect of PSB on germination of sugarcane plants is shown in Table-3. It is evident from the data that association of bacterial isolates significantly increased the germination percentage in presence of rock phosphate. As high as 100% germination was recorded with isolates no 121 followed by 111 and120 (83%). This clearly shows the P solubilization by bacterial isolates has affected the plant germination.

Table 3– Effect of PSB on plant germination

Sl No	Bacterial isolates	Percent Germination	
		Without Rock Phosphate	With Rock Phosphate
1	100	16 5	49 5**
2	101	16 5	33 0*
3	107	16 5	33 0*
4	111	16 5	83 0***
5	113	33 0	66 5*
6	120	33 0	83 0**
7	121	16 5	83 0***
8	150	49 5	66 5
9	151	16 5	33 0
10	control	16 5	

*P<0 05, **P<0 01, ***P<0 001

To evaluate the of P-solubilizing bacterial isolates on plant growth, physiological parameters were recorded at one-month stage. Physiological parameters such as shoot length, number of leaves, plant fresh weight and dry weight showed considerable improvement in PSB inoculated plants. The P-solubilizing bacteria significantly affected all the parameters studied in the presence of RP. As compared to control variable amounts of biomass products were accumulated using different bacterial

ısolates it was maximum ın isolates number 151 (0. 147g), followed by 111 and 150 (0. 121g)(Table-4).

The result of P-uptake by PSB inoculated plants in presence of RP is shown in Table-5. In dried plant samples the concentration of P was determined spectrophotometricaly. Results showed that plants inoculated with isolate number 107 had highest P-uptake (0.95%) followed by isolate number 101;121 and 140 (0.66%, 0.61%, 0.53% respectıvely) in presence of RP. In absence of RP high P-uptake was found in plants ınoculated with isolate number 107 (0.48%)followed by 121 ,120 (0.44%, 0.42% respectively).

Table 4– Effect of PSB on plant growth

Sl No	Bacterıal Isolates	Without Rock Phosphate				With Rock Phosphate			
		Shoot length (cm)	Number of leaves	Fresh weıght (g)	Dry weıght (g)	Shoot length (cm)	Number of leaves	Fresh weıght (g)	Dry weıght (g)
1	100	6 2	2	0 32	0 049	6 9	3	0.36	0 056
2	101	5 7	3	0 30	0 042	5 0	4	0 27	0 05
3	107	4 2	2	0 23	0 033	5 0	3	0 36	0.064
4	111	5.7	3	0 52	0 081	6 0	2	0 77	0 24
5	113	4 2	3	0 43	0 068	6 2	3	0 48	0 109
6	120	9 9	3	0 50	0 101	6 0	3	0 36	0 056
7	121	6 8	4	0 59	0 077	7 0	4	0 72	0 099
8	150	7.0	0	0 40	0 056	5.5	0	0 67	0 121
9	151	6 5	3	0 78	0.139	6 8	3	0 91	0 147
10	control	4.9	3	0 25	0 039	4 7	3	0 19	0 031

Table–5 Effect of PSB on P-uptake

Sl No	Inoculated Bacterial Isolates	Percent Phosphorus Content	
		Rock Phosphate Treated	Without Rock Phosphate treated
1	100	0 48	0 32
2	101	0 66	0 33
3	107	0 95	0 48
4	111	0 24	0 23
5	113	0 39	0 32
6	120	0 49	0 42
7	121	0 61	0 44
8	150	0 53	0 24
9	151	0 42	0 28
10	control	0 46	0 38

Sugarcane endophytic bacteria were studied for PSA and found involved in P solubilization *in situ* studies of these strains for plant -bacterium interaction on sugarcane plants strongly suggest their potential as bio-fertilizer to partially replace phosphatic fertilizers and use low grade rock phosphate (insoluble phosphate) in sugarcane crop.

Acknowledgement

Dharmendra K. Parihar thanks Director, Indian Institute of Sugarcane Research, Lucknow for providing facilities at the Institute.

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Ultrastructure of the Corpuscles of Stannius of a freshwater teleost, *Barbus sarana*

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Abstract

The CS of *B sarana,* contains only one cell type unlike other freshwater teleosts The cells are characterized by large electron dense secretary granules and abundant GER These cells are comparable to type 1 cells of the CS of previously described freshwater species The type 2 cells which are ubiquitous feature of the CS of the freshwater teleosts are absent in *B sarana* We now present evidence that relationship of type 2 cell to freshwater is not exclusive and the presence of type 2 cell is not essential for life in freshwater The darkly stained cells represent apoptotic type 1 cells and point towards a high turnover of these cells

(**Keywords** Corpuscles of Stannius / ultrastructure / teleosts)

Introduction

The corpuscles of Stannius (CS) are unique calcitropic endocrine gland, found exclusively in teleostean and holostean fishes[1]. It is now well established that CS secrete a homodimeric glycoproteinous hormone, the stanniocalcin, which effectively reduces the uptake of calcium from ambient water *via* gills and gut[2,3,4]. These bodies have been shown to contain either one or two structurally different types of cells[5]. Recently, stanniocalcin has also been identified in various tissues of mammals including human, where it functions as a regulator of female reproductive system[6,7]. Thus the hormone, stanniocalcin seems to be of much wider occurrence in vertebrate groups than hitherto believed.

In spite of some work already done on fish CS, the cellular composition of these glands and their mode of secretion are not clearly understood. Data on the fine structure of CS of indigenous fishes is scant[7,8]. Therefore, it was considered pertinent to investigate the ultrastructure of the CS of a freshwater teleost *Barbus sarana* and the findings are reported in this paper.

Materials and Methods

Live specimens of *Barbus sarana* were collected from the river Swarnarekha and were kept in laboratory for acclimatization for ten days. The CS from the kidney were prefixed in Cacodylate (buffered- 0.15M, pH 7.2) gluteraldehyde solution (2%) at room temperature for ten minutes. The tissue was then fixed in similarly buffered solution of 1 % osmium tetraoxide for two hours at 0° C and then embedded in Epon. Ultrathin sections were stained with lead citrate and examined under an electron microscope (Philips EM 300)

Results

B sarana possesses a pair of small Stannius bodies on the peritoneal surface of the posterior kidney. At the ultrastructral level, a homogenous population of cells is apparent. The glandular parenchyma consists of oval cells, characterized by abundant membrane-bound large electron dense granules of varying shapes and diameters, scattered randomly in the cytoplasm (Fig. 1). The granular endoplasmic reticulum (GER) is studded with ribosomes and arranged in lamellar arrays. The cisternae of the network in some cases are filled with secretory materials of moderate opacity. Filamentous mitochondria with lamellar or lobular cristae are also present. A very large nucleus with dense nucleolus is conspicuous (Fig. 2).

Besides these cells, a few other darkly stained cells having similar secretory granules and GER, but displaying different morphology are also evident (Fig. 3). These cells are characterized by multilobate shrunken nuclei separated from their nuclear membrane. The large secretory granules are also separated by wide spaces from their limiting membrane The cells seem to be exhausted as indicated by the appearance of a large number of vacuoles.

Discussion

Earlier works on the CS of freshwater teleosts such as *Gasterosteus aculeatus*[10], *Oncorhynchus mykiss*[11], *Oreochromis mossambicus*, *Fundulus heteroclitus*[12] *Oncorhynchus kisutch*[13] have shown the presence of two structurally different type of cells in these glands. In all species examined so far, the pre-dominant cells - called type 1 cells, are large and rounded in appearance and contain prominent granular endoplasmic reticulum and Golgi areas. They are further characterized by the presence of abundant large electron dense secretory granules. In addition to type 1 cells, a second cell type (type 2) has been noticed. They are usually polygonal in

shape and possess long cytoplasmic processes. The granular endoplasmic reticulum is scarce as are the Golgi areas The secretory granules are also very small and few in number[5]. Till date, type 2 cells have been reported only from freshwater fish species. These cells have not been described in marine teleosts, suggesting that type 2 cells may be producing a hormone specific for life in freshwater

The ultrastructure of the gland cells of the CS of *B sarana* is similar to that of type 1 cells of other freshwater teleosts described earlier. The type 2 cells, which have been ubiquitously present in the CS of freshwater species reported so far, are absent in *B sarana*

Recently, a homogenous population of type-1 cells only has also been reported in the CS of another freshwater teleost *H fossilis*[8,9]. In teleosts, the type 1 cells have already been established as the source of stanniocalcin, the principal hormone of CS because these cells respond with reduced activity to an increase in the calcium concentration of the ambient water and enhanced activity when the conditions are reversed[14]. The nature of type 2 cells is unclear. Several authors have considered these cells as a specific cell type, functionally different from type 1 cells producing an unknown hormone. Many arguments are in favour of this view; first, the shape and ultrastructure of type 2 cells are completely different from those of type 1 cells and transitory stages have not been recorded Secondly, in contrast to type 1 cells type 2 cells do not respond with increased or decreased secretory activity to changes in the calcium concentration of the ambient water[11,14]. Thus type 2 cells have not been established as a distinct functional cell type. Many observation on the other hand lend support to an alternative interpretation i.e. type 1 and type 2 cells are structurally different forms of only one functional cell type, and produce the same hormone. Immunocytochemical tests have demonstrated that both cell types specifically bind to antibodies raised against secretory products of CS[15]. Furthermore, biochemical studies on the CS of tilapia, trout and eel have presented evidence for the presence of only one secretory pathway in the CS with stanniocalcin as single final product[16].

From the present observations on *B. sarana* it appears that the CS of all freshwater teleosts do not necessarily possess two different types of cells and also that type 2 cells are not typical cell type which are essential for life in freshwater, as was previously suggested. The interpretation of type 2 cells as a specific cell type producing a hormone essential for life in freshwater is no longer tenable.

The darkly stained cells, observed amidst the cellular population in the CS of *B. sarana* are clearly related to type 1 cells, as indicated by the presence of large secretory granules and extensive GER in them (Fig. 3). Similar dark cells have also

been described earlier as artifacts[17,18,19]. We suggest that these dark cells represent apoptotic degeneration[20] of type 1 cells rather than a specific cell type. The. apoptosis represents the physiological mode of cell death. The sequential phases of apoptosis are progressive cytoplasmic and nuclear densification, widening of the nuclear envelope, secretory granular membranes and GER membrane and this also applies to the dark cells in CS of

B sarana The occurrence of apoptotic stages of type 1 cells is suggestive of a high state of turnover in these cells. This interpretation is supported by observation of Meats *et al.*[11] as the number of dark cells increase in *Salmo gairdneri* when the secretory activity of the corpuscles is stimulated by transfer from freshwater to seawater.

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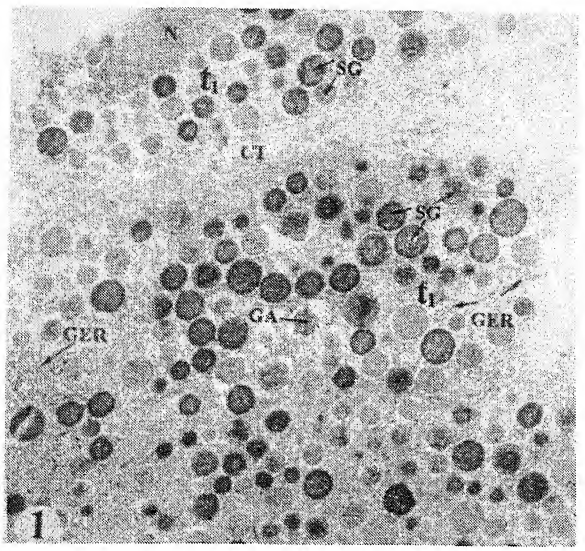

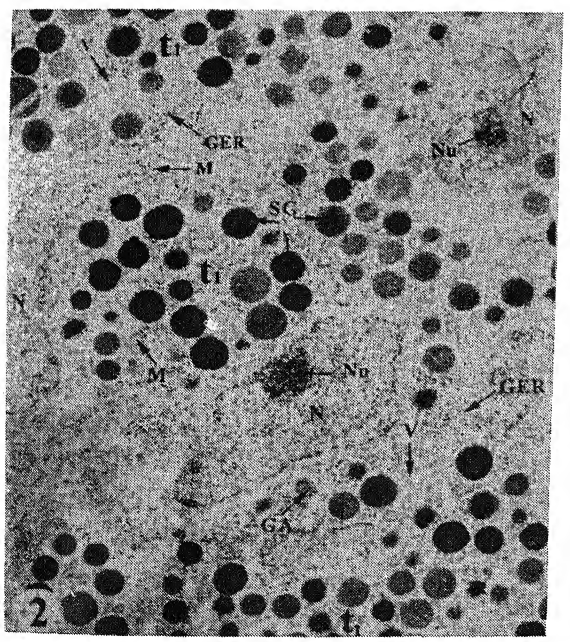

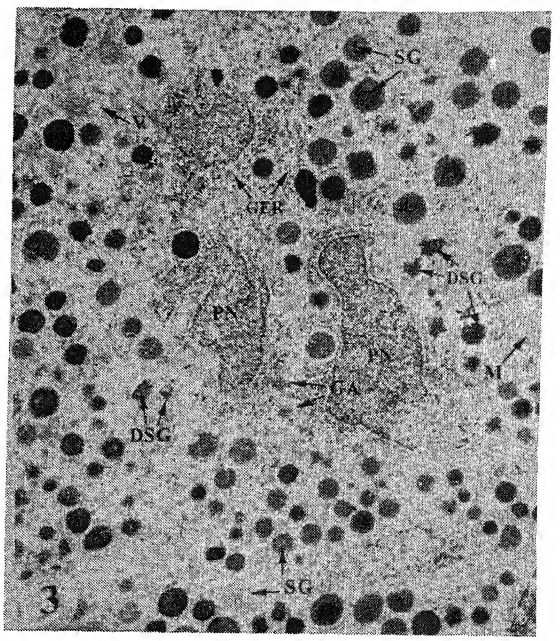

Fig. 1–Scretory cells of the CS of *B. sarana,* showing homogenous cellular population of type 1 (tl) cells, large nucleus (N), abundant granular endoplasmic reticulum (GER), Golgi apparatus (GA), intercellular connective tissue (CT) and numerous large secretory granules (SG) (x 12600)

Fig. 2–An enlarged portion of Stannius cell (tl) of *B. sarana,* showing large nucleus (N) with dense nucleolus (Nu), filamentous mitochondria (M), Golgi apparatus (GA) with pre-secretory material, large darkly stained granules (SG), and abundant granular endoplasmic reticulum GER) (x26400)

Fig. 3–Darkly stained shrunking apoptotic cells in the CS of *B. sarana* with pycnotic nuclei (PN), disintegrating secretory granules (DSG), vacuoles (V), condensed granular endoplasmic reticulum (GER) and shrunken mitochondria (M) (x 26400)

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Protective role of vitamin E in amelioration of radiation induced damages in chick testis

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Abstract

The present investigation pertains to a study of the protective influence of vitamin E on the testis of leghorn chick (*Gallus domesticus*) subjected to 5 6 Gy whole body gamma irradiation, The study was conducted on 7 days old chicks for a maximum period of 60 days postirradiation Alterations in mortality rate, body weight and testes weight along with radiation-induced changes in the histological profile of the testes, have been considered for the study The results reveal definite radioprotective role of vitamin E on the growth, structure and regeneration of the testicular, tissue subjected to radiation insult

(**Keywords :** vitamin E/ chicks/gamma radiation /testes)

Introduction

A number of chemical agents tested for radioprotective effect have been found unsuitable for clinical radiotherapy because of their toxicity at the effective doses. Presently, the emphasis is on natural products that are not toxic at the effective dose level. Vitamin E as an antioxidant, has received much attention as it is associated with biomembranes and protects them by neutralizing oxidation induced free radicals[1]. Its administration at high doses is safe. Doses of 200 - 2400 IU vitamin E per day have been administered for periods up to four and a half years in clinical trials and have not shown any adverse effect[2]. Testis is a radiosensitive organ and is considered a very important parameter for assessing radiation damage. Radiosensitivity varies with age, younger animals being more radiosensitive than the older ones. In the light of above, the present investigation was undertaken to study the radiation induced alterations in the testes of growing immature chicks and its modulation with vitamin E pretreatment.

Materials and Methods

Newly hatched white leghorn male clicks *(Gallus domesticus)* were procured from Govt. Hatchery, Sunder Nagar. These were maintained in optimal animal house conditions. Feed (Hindustan Levers) and water were given *ad libitum.*

Seven days old male chicks were divided into four groups. The animals of group A served as untreated non-irradiated controls whereas the chicks in group B were given vitamin E (d-∝-tocopherol -Sigma) orally @ 1mg/g body weight. Animals of groups C and D were exposed whole body to a dose of 5.6 Gy gamma radiation at the rate of 0.2 Gy/sec. in BARC gamma chamber 900 with autotimer The dose rate was determined by using Fricke's dosimetery[3]. Animals of group D were given vitamin E three hours prior .to irradiation., @ 1mg/g body weight. Animals were sacrificed by cervical dislocation on days 1,3,5,7,14,28,35, and 60 days postirradiation. At least six animals were sacrificed at each interval. Testes were excised from sacrificed animals, weighted, fixed in bouin's fluid and processed for histological studies. Paraffin embedded tissue sections of 5μm thickness were cut and stained with harris hematoxylin and eosin. The number of primary - spermatocytes were counted in atleast 300 cross sections of seminiferous tubule. The diameter of the tubular cross sections was measured using stage and ocular micrometer. Mortality, body and testes weights of the chicks were recorded at each postirradiation interval.

Results

Radiation Mortality : Chicks subjected to 5.6 Gy showed mild signs of radiation sickness. Mortality was 60% during the 30days posirradiation period in the untreated chicks and 22% of the chicks died with in 24 hours of irradiation. In the vitamin treated irradiated chicks, the mortality was 13.3% with in 24 hours and 40% during the 30 days of observation.

Body and Testes Weight (Table 1; Graphs 1-2 Figs. 1-2) : Body weights of the chicks increased from 55.6±0.46g to 305.0±728 g during 60 days of normal growth. Irradiation decreased the body weight and it was 30.2% less than that of the control at 35 days. At 60 days the body weight of irradiated chicks was 20.9% less. In comparison to irradiated chicks, the change in growth and body weight of vitamin treated chicks was less marked throughout the experiment and 11.4% higher body weight was recorded at 60 day stage as compared to untreated irradiated chicks.

Weight of !he testes in chicks decreased following irradiation and was more marked in the later period. Maximum decrease (57.2%) was noted at 35 day stage. In the vitamin E fed irradiated chicks, loss of weight of testes was less in comparison to untreated irradiated chicks which was very much evident at later stages. At day 35 post irradiation, a decrease of 39% in weight of the testes was observed. The testes-body weight ratio was higher in the vitamin treated irradiated chicks at days 35 and 60 post irradiation as compared to the untreated irradiated chicks.

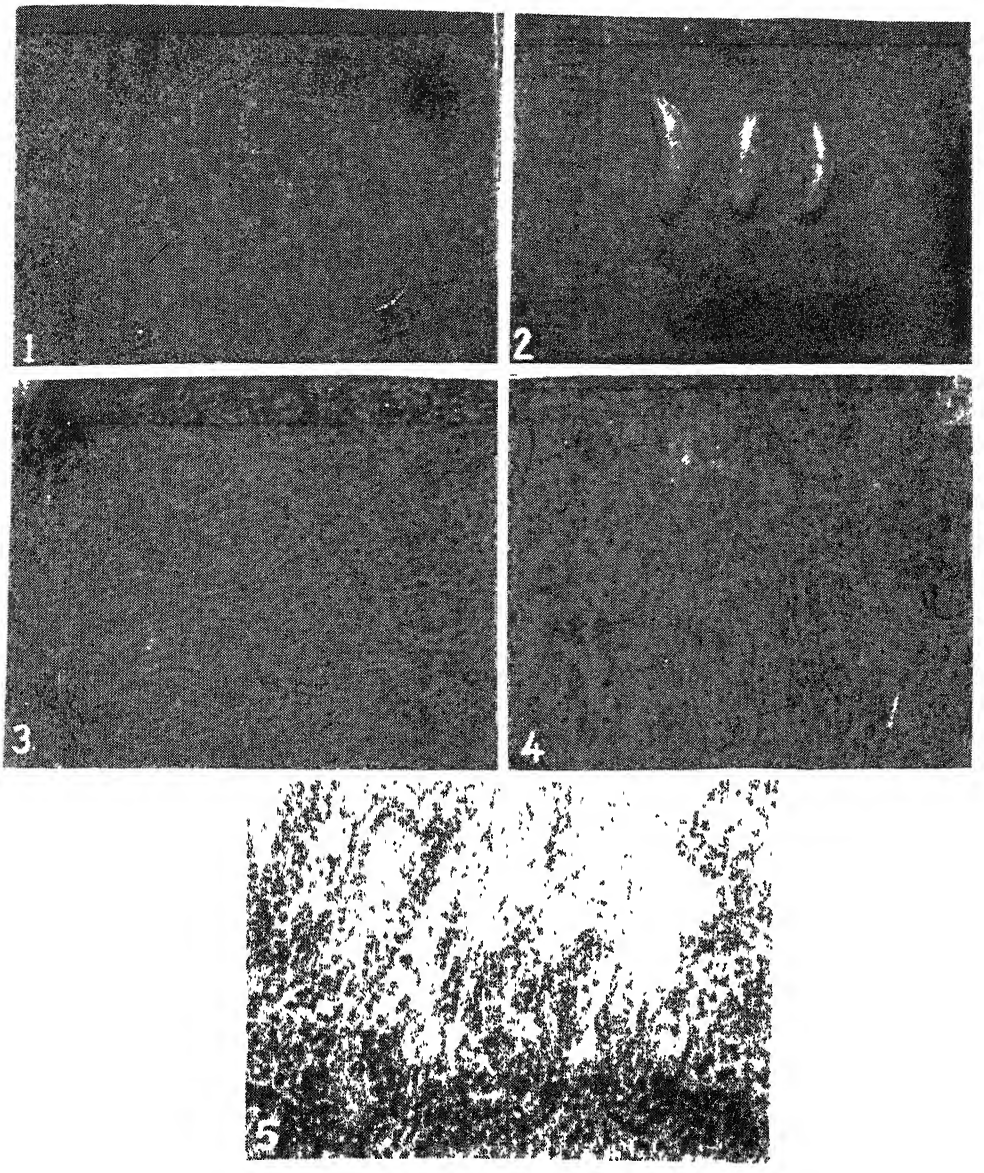


Fig 1–Photograph of testis of normal (a) vitamin E fed (b) untreated irradiated (c) and vitamin E treated irradiated (d) chicks at 28 day stage

Fig 2–Photograph of testis of normal (a) vitamin E treated irradiated (b) and untreated irradiated (c) chicks at60 day stage

Fig 3–T S of normal chick testis at 3 day stage showing outer capsule, seminiferous tubules (ST) lined with a single layer of spermatogonia cells Note the loosely arranged interstitial cells (IC) between the tubules X 150

Fig 4–T S of testis at 3 days postirradiation exhibiting shrunken condition of the tubules Note the infiltration of erythrocytes in the tissue (↑) XI50

Fig 5–T S of vitamin treated chick testis at 3 days postirradiation showing less damage X500

Fig 6–T S. of normal chick testis at 28 day stage. X 150

Fig 7–T.S of testis at 28 days postirradiation showing shrunken condition of the seminiferous tubules and dead cells in some of the tubules X150

Fig 8–T S of vitamin treated irradiated testis at 28 days postirradiation The tissue architecture is less damaged X150

Fig 9–T.S of normal testis at 28 day stage exhibiting gonial cells arranged at the periphery of the tubules X500

Fig 10–T S of irradiated chick testis at higher magnification. Note the shrunken condition of the tubules. X500

Fig. 11–T S of vitamin treated chick testis at 28 days postirradiation. Note the normal appearance of seminiferous tubules X500

Table 1– Changes in body weight, testes weight and testes weight to body weight ratio of 7 days old chicks after exposure to 5 60 Gy gamma radiation with or without vitamin E treatment

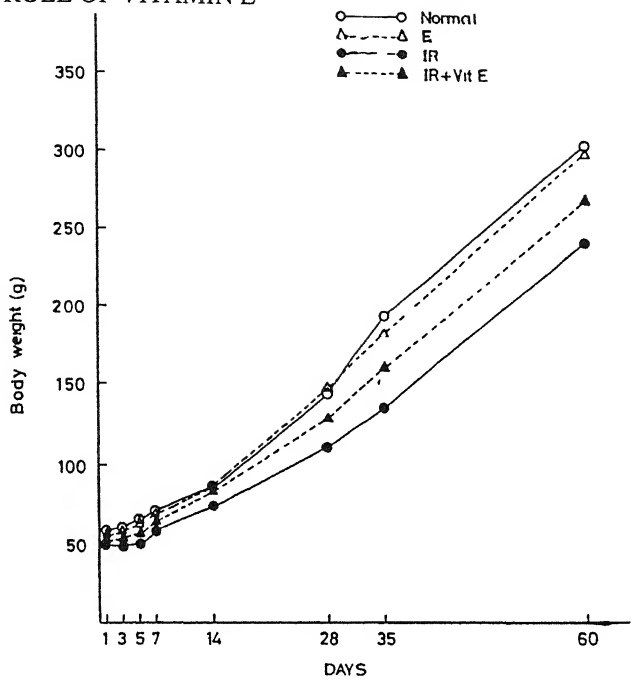
Post Irradiation (Days)	Groups	Body Weight (g)	Testes weight (mg)	Testes body weight ratio
	Normal	55 6 ± 0.46	11 3 ± 0 23	0 00020
	Vitamin E	54 3 ±1 15	11 5 ± 0 15	0 00020
1.	Irradiated	50 8 ±0.98	10 5 ± 0 28	0 00021
	Irradiated	51 4 ± 0.99	11.1 ± 0 16	0 00022
	+Vitamin E			
	Normal	57 8 ± 1 36	11 8 ± 0 44	0 00020
	Vitamin E	55 8 ± 1 99	11 2 ± 0 08	0 00020
3	Irradiated	47.6 ± 0.75	9 6 ± 0 21	0 00020
	Irradiated	52 9 ± 1 21	10 8 ± 0 16	0 00020
	+Vitamin E			
	Normal	64 0 ± 0 92	12.1 ± 0.17	0.00019
	Vitamin E	63.7 ± 2 50	12 2 ± 0.24	0 00019
5	Irradiated	49 7 ± 1 12	9 8 ± 0.28	0.00020
	Irradiated	56 5 ± 1 47	11.3 ± 0.33	0 00020
	+Vitamin E			
	Normal	70 .4 ± 1 35	15 8 ± 0 44	0.00022
	Vitamin E	69 5 ± 2 50	14 9 ± 0 32	0 00021
7	Irradiated	56.6 ± 1 40	10 5 ± 0 28	0 00018
	Irradiated	63.8 ± 2 37	11 5 ± 0 68	0.00018
	+Vitamin E			

Table 1 Contd...

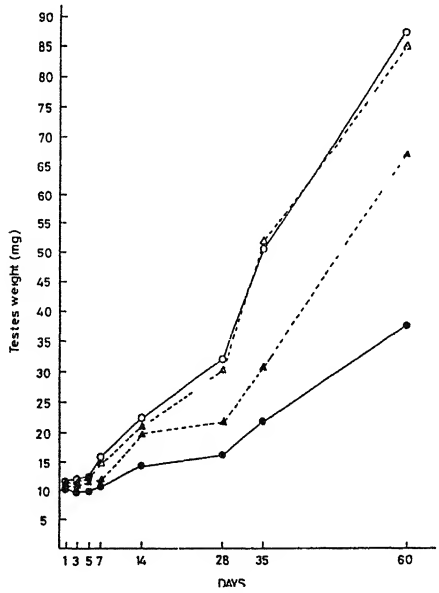
Table 1 Contd

	Normal	86 5 ± 2 47	22 3 ± 0 87	0 00026
	Vitamin E	86 7 ± 2 00	20 8 ± 0 26	0 00023
14	Irradiated	72 1 ± 2 23	14 1 ± 0.39	0 00019
	Irradiated	85 0 ± 2 02	19 6 ± 0 88	0 00023
	+Vitamin E			
	Normal	145 0 ±2 31	32 0 ± 0 28	0 00022
	Vitamin E	146 0 ±1 21	30 0 ± 0 33	0 00020
28	Irradiated	110 0 ± 1 58	16 0 ± 0 88	0 00015
	Irradiated	129 0 ± 1 58	21 6 ± 0 25	0 00017
	+Vitamin E			
	Normal	192 1 ± 3 25	50.5 ± 0 76	0 00026
	Vitamin E	185 9 ± 2 28	51 8 ± 0.34	0.00027
35	Irradiated	134 0 ± 2 92	21 6 ± 0 16	0 00016
	Irradiated	160 0 ± 3 28	30 8 ± 0 44	0 00019
	+Vitamin E			
	Normal	305.0 ± 1 28	87.5 ± 4 38	0.00029
	Vitamin E	300 0 ± 2 37	85.4 ± 3 96	0 00028
60	Irradiated	241 2 ± 2 7	37 5 ± 2.19	0 00015
	Irradiated	268 7 ± 2 44	67 5 ± 2 69	0 00025
	+Vitamin E			

Histology (Figs 3-14) : The paired testes of the fowl are internal. Testis is contained within its connective tissue capsule - the tunica albuginea, which is very thin and does not give off septa to divide the testis into separate lobules. The seminiferous tubules of the prepubertal males are few during early stages. They are



Graph 1–Graph showing changes in body weight of 7 days old male chicks after whole body exposure to 5 6 Gy gamma radiation with and without vitamin E treatment.



Graph 2–Graph showing changes in testes weight of 7 days old male chicks after exposure to 5 6 Gy dose of gamma radiation with and without vitamin E treatment.

lined with a single layer of spermatogonial cells. The interstitial tissue between the tubules is abundant and loosely arranged. From 14 days onwards the population of gonial cells increases and at 35 day stage, large number of spermatogonial cells and a few primary spermatocytes are observed in the tubules. A gradual increase in the cross sectional dimensions of the seminiferous tubules is observed and at 60 day stage, the testes architecture becomes more compact with the reduction of interstitial tissue. The diameter of the tubule in the normal testis at60 day stage was 73.62 ± 0.80 μm and the number of primary spermatocytes per tubule cross section was 15.47 ± 0.27. In the vitamin fed chicks of group B, histological profile was similar to that of the normal testis and diameter of the seminiferous tubule measured was 70.32 ± 0.38 and the number of spermatocytes per tubule cross section as 16.21 ± 0.46.

Histopathological changes were observed in the irradiated testis. The damage was less marked in the early postirradiation period. During the initial intervals, testis appeared smaller in size compared to the normal. The seminiferous tubules appeared shrunken The interstitial cells were intact, though infiltration of red blood cells in the intertubular tissue was distinct and hyperaemia was prominent. In some of the tubules, spermatogonial cells got detached from the basement membrane and exfoliated into the lumen. At days 28 and 35 postirradiation, the damage was prominent. There was significant regression of the tubules, distortion and disorganisation of germinal epithelium was noticeable and some of the germinal cells showed nuclear pyknosis. Recovery of the tissue was evident at day 60 postirradiation but population of germ cells and the diameter of seminiferous tubules remained subnormal. The diameter of the seminferous tubule (56.98 ± 0.73 μm) and the number of primary spermatocytes per tubule cross section (11.96 ± 0.33) in the irradiated testis were significantly less ($p < 0.001$) in comparison to that of the normal non-irradiated testis.

In the vitamin E treated irradiated chicks, damage to the tissue was comparatively less in the early stages. Seminiferous tubules exhibited shrunken condition but to a lesser extent compared to the untreated irradiated testis as was evident from the histological profile of the treated testis. However, some of the tubules in the central region showed disorganized epithelium and exfoliated cells were visible in the lumen of some tubules. The damage was more in the later intervals, the population of germinal cells decreased and some of the gonial cells showed pycnotic nuclei. At day 35 postirradiation, the vitamin treated testis depicted some recovery. The basement membranes of the tubules and the tissue architecture appeared normal but the population of spermatogenic cells remained subnormal. The recovery was clearly seen in the treated testis at 60 days postirradiation. The seminiferous tubules were closely packed like the normal testis. The diameter of the tubule was 69.08 ± 0.73 μm. The

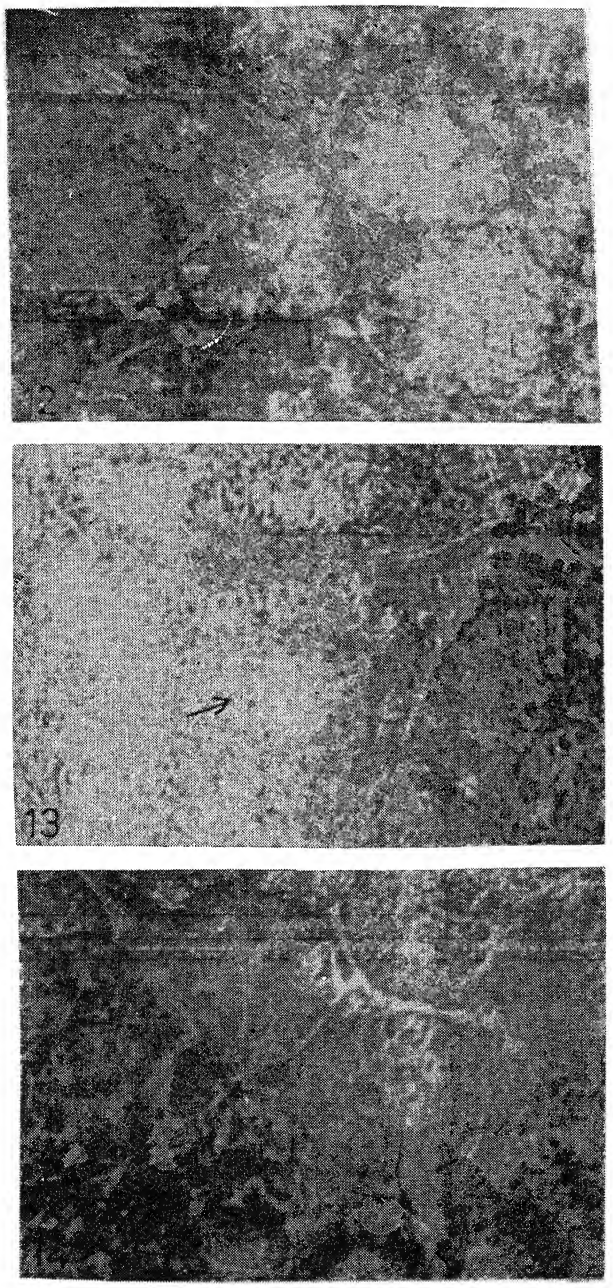


Fig. 12–T.S. of normal chick testis at 60 day stage showing compactly arranged tubules. X500

Fig,13–T.S. of chick testis at 60 days postirradiation. Note the diameter of the seminiferous tubule (↑) which is still subnormal. X500

Fig,14–T.S. of vitamin treated chick testis at 60 days postirradiation exhibiting normal histological profile. X500

number of primary spermatocytes per tubule cross section was significantly higher (14.51 ± 0.26; $P < 0.001$) than seen in the untreated irradiated testis.

Discussion

The mortality in chicks during 30 days postirradiation period appears to be on account of haemopoietic syndrome. The 22% mortaliy within 24 hours is probably due to vascular damage as reported earlier[4,5]. Damage to the microcirculation is not only caused by high dose of ionizing radiations but also at low dose[6].

The increment rate of body weight declined after exposure to 5.6 Gy gamma radiations. The loss in body weight is a good indicator of radiation injury. Anorexia and water loss from the body appears to be responsible for the decreased body weight because of the injury to the intestinal wall and due to wide spread hemarrhages[7]. The decrease in testicular weight might have been caused due to the killing of germ cells after exposure and their removal from the testicular capsule[8]. Similar results showing loss in weight of testes have also been reported by some earlier workers[9,10]. Severe injury to the tissue was observed during 28-35 days after exposure. This could be attributed to the depletion of spermatogonia on account of inhibition of mitosis in spermatogonial cells as well as cell killing effect of whole body irradiation. Of all the spermatogonial elements, differentiating spermatogonial cells are most susceptible to radiation effects[11,12]. Such a late effect is on record[13,14]. The stem cells are more radio-resistant and those cells that escape radiation injury restore spermatogonial population at a later stage. This is evident at 60 days postirradiation where regeneration of the testis although incomplete was clearly visible.

The histological profile of vitamin treated irradiated testis exhibited less damage. The shrunken condition of the testis was lesser in degree in the initial postirradiation period as compared to the untreated irradiated testis. Regeneration was observed at 60 days after irradiation. Comparison of the diameter of seminiferous tubules clearly shows that vitamin treatment had restricted their shrinkage. The restoration of tissue architecture and the increase in the number of primary spermatocytes nearer to the control value clearly suggests the protective influence of vitamin E to the testicular tissue. With the passage of time the increase in the body weight and testis weight of the vitamin treated irradiated chicks over the untreated irradiated chicks indicate faster recovery of the radiation damage.

Free radical production and lipid peroxidation are potentially important mediators in tissue damage. An increase of 58.6% in the level of lipid peroxidation in the liver of chicks has been reported at day 1 after exposure to 5.6 Gy dose, which got reduced to

32.6% ($p<0.001$) with vitamin E pre-treatment[15]. The physical state of the membrane (fluidity) during irradiation plays an important role in determining the levels of injury and repair following exposure[16]. Vitamin E inhibits destructive peroxides of polyunsaturated lipids and reacts as a chain-breaking antioxidant and thus helps maintain the stability and structural integrity of the membrane systems[17]. Enhanced stem cell survival with vitamin E treatment has also been indicated[18,19]. It might have been a contributory factor in bringing about faster recovery of the treated testis.

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Prophylactic role of vitamin E against radiation induced biochemical alterations in the liver of Swiss albino mice

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Abstract

Adult Swiss albino mice were exposed to 4 80 Gy gamma radiation in the presence (experimental) or absence (control) of Vitamin E (α-tocopherol) These animals were sacrificed at various autopsy intervals from 6 hrs to 20 days In control animals, an elevation in glycogen and protein content was found till day 2 Soon after this, their level decreased at consecutive intervals and reached to near normal on day 20 of post-treatment

On the contrary, a continuous decrease in cholesterol concentration was noted after exposure to 4 80 Gy dose of radiation and it was found to be minimum at day 2 but later it increased, however, the normal level did not obtain even till the last day of irradiation (i e 20 day) A similar pattern of biochemical alteration was exhibited by experimental group (Vitamin E + 4 80 Gy), but to a lower extent A normal level of glycogen and cholesterol was regained on day 10 in this group, while it was on day 20 in the case of control group

(**keywords** . Swiss albino mice/gamma radiation/vitamin E/glycogen/protein/cholesterol)

Introduction

With the development of nuclear technology, radiation have become an inevitable part of modern civilization. Exposure come from detonation of nuclear weapons, travel in outer space nuclear powered vehicles, extensive use of radiation and radioisotopes in medical diagnosis and therapeutic purposes. In contrast to other forms of radiation, ionizing radiation has the capacity to break chemical bonds; it imparts energy to living cells though random interaction with atoms, giving rise to ions and reactive radicals. These, in turn, cause molecular changes that may lead ultimately to biological injury[1].

In recent years, an extensive research work has been carried out in the field of chemical radio-protection. Some chemicals have been tested for their protective ability against ionizing radiation and found to be promising, but their practical

applicability is limited. Vitamins, a group of complex organic compounds present in minute amounts in natural foodstuffs, are essential to normal metabolism in living beings. Some act as co-factor in enzyme system and others as antioxidants or antagonists. These compounds have generated a great deal of interest in recent years for a wide range of protective effects in biological systems[2-4]. In light of the above, the present study is an attempt to assess the effect of Vitamin E (α-tocopherol) on the liver of Swiss albino mice subjected to sublethal whole-body gamma radiation.

Materials and Methods

Young-adult Swiss albino mice, 6-8 weeks old with an average body weight of 22±3 gm., were selected from an inbred colony and maintained on standard mice food and water *ad libitum*. These animals were divided into three groups Animals of Group -I (vehicle treatment) and II (experimental) were administered peanut oil and Vitamin E (25 mg/Kg b.wt. in peanut oil) respectively, orally once in a day for 7 consecutive days. Group - III (control) received an equal amount of peanut oil, treated in the same manner as the drug solution (Vitamin E). After 1 hour of above treatments on 7th day, animals of the groups II and III were exposed to gamma radiation from a Co^{60} source. A minimum of the six animals were sacrificed from above groups at various post-treatment autopsy intervals viz. 6 hrs., 12 hrs., 1 day, 2 days, 5 days, 10 days and 20 days.

For the biochemical estimation, liver was collected from autopsied animals and the homogenate was prepared. Glycogen, protein and cholesterol levels were measured by Montgomery[5], Lowry *et. al.*[6] and Leiberman[7] methods respectively. Spectrophoto-meter (Systronix UV-vis Spectrophotometer 108) was used to measure the optical densities. All the results were expressed as mean±S.E., and the degree of significance was determined by the Student's 't' test.

Results and Discussion

Various biochemical constituents like glycogen, protein and cholesterol in liver exhibited a non-significant variation with respect to normal (without treatment) in peanut oil treated mice of Group - I (vehicle treatment).

After 4.80 Gy irradiation, glycogen levels was found to be elevated (50.94% above normal) at the first autopsy interval (6 hrs.). The increasing pattern persisted and the peak was measured on day 2, where the level was almost 2 folds higher than the normal. Later autopsy intervals revealed a declining trend, and on day 20, almost a normal value was regained.

The variations in liver glycogen level in Vitamin E treated animals was visualized as similar to control (4.80 Gy irradiation) but the degree of increase was found to be much lower. The normal value was restored in this group on day 10 unlike day 20 in the case of control (Table - 1).

An elevated level of glycogen contents in the present study may be due to the increasing energy requirement of degenerating and aberrent hepatic cells. The elevation in glycogen concentration decreased at later period of study which may be due to the recovery in cell population at such post-irradiation intervals. The lower concentration of glycogen in the Vitamin E treated group (experimental) in comparison of corresponding controls too demonstrate the same situation. It is also possible that the decreased glycogen concentration might lead the depression in energy reserves which resulted in the degeneration of hepatic cells.

Protein content increased to about 34 per cent in comparison to normal at 6 hrs. autopsy interval. It raised further by reaching to the highest level (61 24% of normal) after 2 days of irradiation. Thereafter, the protein level tended to recover on later autopsy intervals, but the normal range could not be measured even till the end of experimentation (i.e. day 20, Table - 1).

Mukerjee and Goldfeder[8] suggested that a significant rise in protein synthesis may be due to the increased transport of amino acid through the plasma membrane as a consequence of permeability change in irradiated cell membranes They also reported that a considerable increase in the number of ribosomes may occur due to their increased mobilisation from endoplasmic reticulum, and it leads to the increased protein synthesis. This may be one of the reasons for the elevated levels of protein in the present investigation.

Vitamin E treated animals prior to irradiation showed a significantly lower concentration of protein in liver than the control (Table - 1). The maximum level of it was observed on day 2 after irradiation. Chemical repair of the radiation induced DNA radicals by hydrogen atom donation[9] may be an important mechanism of protection against radiation -induced protein denaturation in liver of mice.

After 4.80 Gy radiation exposure, cholesterol level was found to be significantly lower than the normal throughout experimentation. On day 2, it depleted by 12.72 per cent in comparison to control, but later increased at consecutive intervals without reaching to normalcy (Table - 1). The reduction observed in cholesterol concentration during early intervals in present study might be due to the stress response caused by irradiation which stimulates the synthesis of steroid hormones via hypothalamic-pituitary system.

Table 1– Variations in various biochemical constituents in liver of mice after 4 80 Gy gamma exposure in the presence (experimental) or absence (control) vitamin E

Biochemical Parameters	Treatment	Post – Treatment Autopsy Intervals						
		6 hrs	12 hrs	1 day	2 days	5 days	10 days	20 days
Glycogen	C	6 37±0.130●	6 83±0 134●	7 44±0.190●	8 32±0 268●	7 76±0 132●	6 57±0 282●	4 28±0 083
(4.22±1 30 mg/gm)*	E	5 42±0 284□	5 90±0 159□	6 51±0.397	7 00±0 226□	5 51±0 228●	4 25±0 262●	4 20±0 139
Protein	C	200 24±4 742●	205 68±2.344●	227.46±3 829●	240 88±7 243●	200 22±3 787●	170 67±1.992●	155 09±2 867
(149 39±3 23 mg/gm)*	E	163 93±438●	170 98±5 952●	183.02±8 500●	192 62±7 243●	157 56±7 575●	153 80±8 769	147 40±308
Cholesterol	C	4 05±0 03●	3 97±0 02●	3.90±0.02●	3 84±0 04●	3 90±0 05●	4 28±0 04♦	4 36±0 05
(4.40±0 03 mf/gm)*	E	4 27±0.04□	4 23±0.05●	4 16±0 01●	4 00±0 01□	4 04±0 06	4 37±0 04	4 40±0 01

*—Figures in parenthesis represent the values in normal mouse (without treatment)

C—Control (4 80 Gy irradiation)

E—Experimental (Vitamin E + 4 80 Gy irradiation)

♦ P < 0 05

□ P < 0 01

● P < 0.001

In vitamin E protected group, cholesterol level significantly declined upto day 2, where it was found to be 3 63 per cent higher than the respective control. Day 2 onwards, it elevated and reached to normal level by the last autopsy interval (i e. 20 days) The increase levels of cholesterol in Vitamin E pre-treated animals has been reported to be attributable to its decreased utilization for steroidogenesis, which may be attributed to pituitary inhibition or a direct inhibitory action of the target tissue[10] These findings duly support the results obtained from the present study.

Glutathione (GSH) is a versatile protector and executes its radioprotective function through free radical scavenging, restoration of the damaged molecules by hydrogen donation, reduction of peroxides and maintenance of protein thiols in the reduced state[11] The depletion of GSH in both blood and liver as observed in the present study[12] is one of the main causes of radiation induced hepatic toxicity, and Vitamin E helps in restoring GSH level, thus offering a significant protection against radiation induced injury (Table-2). Therefore, the present investigation demonstrates that application of Vitamin E prior to sub-lethal dose of gamma radiation is quite potential to provide protection against biochemical hepatic lesions induced by irradiation.

Table 2– Glutathione (GSH) level in Swiss albino mice after 4 80 gamma irradiation in the presence or absence of vitamin E

Treatment	GSH level (mean + S E)	
	Blood (μg/ml)	Liver (μg/ml)
Normal (Sham irradiation)	3 94±0 196	6 89±0 187
Vitamin E	5 21±0 274□	8 28±0 226□
4 80 Gy	2 76±0 176□	5 64±0 213□
Vitamin E+4 80 Gy	3 80±0 1 01□	6 70±0 150□

Significance level □p<0 01

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Immunocytochemical localization of neurophysin, neuropeptide Y and serotonin in the pseudobranchial neurosecretory cells of two catfish species *Clarias batrachus* and *Heteropneustes fossilis*

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Abstract

The pseudobranchial neurosecretory cells of fish gills share several morphofunctional features with the paraneuronal cells of respiratory tract of vertebrates In the present investigation, an immunohistochemical staining technique is used to find out the nature of secretory material present in the cells of the pseudobranchial neurosecretory system, in two Indian catfish species, namely *Clarias batrachus* and *Heteropneustes fossilis*, using antisera against neurophysin, neuropeptide Y, serotonin and β-endorphin. The cells in both species were found to be immunoreactive with first three antisera and non-immunoreactive with β-endorphin. The findings are described and compared with immunoreactivity observed in examples of other paraneuronal cells, found in various organs of vertebrates To our knowledge this is the first demonstration of neurophysin, neuropeptide Y and serotonin in the pseudobranchial neurosecretory cells of catfishes

(**Keywords** . neurosecretion/pseudobranchial neurosecretory cells/ antisera/paraneuronal cells/ immunocytochemistry)

Introduction

The occurrence of specialised structures composed of amine and peptide hormone containing cells, within respiratory tracts of various vertebrates is well known [1] These cells have many features in common with the cells of the APUD series, according to the description of Pearse[2], and have been found to contain several biogenic amines and regulatory peptides. These cells, called endocrine like cells[3] have been referred to as bronchopulmonary paraneurons by Fujita *et al.* [4] These paraneuronal cells are the source of neurologically active substances typical of the endocrine cells belonging to the diffuse neuroendocrine systems scattered throughout the animal body. In the mammalian[5,6] and submammalian vertebrate air ways[7-9] including air breathing

fish[10,11] investigated immunocytochemically, among bioactive substances reported, serotonin is the most commonly identified neuroendocrine marker found in neuroepithelial (NE) cells.[11,12,13,14] In fish species investigated so far, immunocytochemical analysis demonstrated that neuroendocrine cells are rich source of bioactive compounds like serotonin, neuron specific enolase, enkephalin, including neuroactive substances of the endothelin type. [13,14,15]

Regulatory peptides observed immunocytochemically in the neuroendocrine cells of fish gill include leu-5-enkephalin, met-5-enkephalin and neuropeptide Y. [16,17,18] An Indian catfish *Heteropneustes fossilis* has been found to be containing calcium binding/regulatory peptides like calbindin D28K, calmodulin and S-100 in its neuroendocrine cells of the gill. [13,14,19] Recent immunocytochemical investigation carried in this Indian species have revealed the presence of endothelin peptides in the neuroendocrine cells of the gill.[20] Co-localization of endothelin with neuron nitric oxide synthase (nNOS) and that of serotonin with neuropeptides has been observed in the neuroendocrine cells of *Heteropneustes fossilis* and *Notopterus chitala*.[21]

Similar neuroendocrine cells are found in the gill region of certain Indian teleostean groups including catfishes close to pseudobranch/carotid labyrinth and the first two efferent branchial vessels.[23] These cells form a diffuse neuroendocrine system the pseudobranchial neurosecretory system of fishes[22,24] and have all the attributes of "paraneuron"[25] and like all the paraneuronic cells they are expected to secrete bioactive/neurologically active substances.

Experimental investigations have revealed association of this system with surfacing behaviour, commonly displayed by these fishes[22,24] suggesting their sensitivity to the level of oxygen in the water. In order to characterise the substances secreted by these cells, histochemical investigations were carried out in which the pseudobranchial neurosecretory cells showed positive reaction with both the argyrophil as well as argentaffin silver staining methods used for identification of endocrine like cells, producing biogenic amines and polypeptides.[26]

In an attempt to reveal immunoreactive characteristics of secretory substances of these cells of pseudobranchial neurosecretory system present investigation was undertaken in two Indian catfish species namely *Clarias batrachus* and *Heteropneustes fossilis*.

Materials and Methods

The fish of either sex weighing between 25-35 g were used in the present study. Fish were anaesthesized with MS222 (Sandoz), perfused transcardially with cold

phosphate buffer and then with Zamboni's fixative. The palate of the fish was exposed to take out the tissue from the gill region adjoining the first two efferent branchial vessels and lateral dorsal aorta connecting the two. The tissue was post-fixed in fresh fixative for 12 hours and then cryoprotected overnight with 30% sucrose solution in phosphate buffer, both at 4°. It was rapidly frozen with 1.5% PVP (polyvinyl pyrolidone) solution and 15 μm thick sections were cut with a cryostat. The sections were mounted on poly-L-lysine coated slides, air dried and stored at –20° until stained.

The sections were rehydrated in tris buffer (pH 7.4) and processed by unlabeled antibody peroxidase anti-peroxidase (PAP) method of Sternberger *et al.*[27] The sections were pre incubated with 10% normal goat serum, followed by the primary antisera against neurophysin, neuropeptide Y, serotonin and β endorphin (Immuno Nuclear Corp.) each at a dilution of 1:1000 for 24-35 h at 4° in a moist chamber. All the dilutions and rinses were carried out in 0.1 M, tris HCl buffer containing triton X 100 (pH 7.4). The sections were then incubated in goat anti-rabbit IgG (National Institute of Immunology, New Delhi, India) for 60 min at room temp., washed in two changes of tris buffer followed by incubation in the PAP complex (Sigma) at 1:100 dilution Sections were then rinsed in tris buffer followed by a 5 min. wash in 0.05 M tris buffer (ph 7.6). and were then treated with 0.05%, 3,3' diaminobenzidine tetra hydrochloride (DAB; Sigma) with 0 03% H_2O_2 in 0.05 M tris buffer (pH 7.6) for 5 to 10 minutes. Slides were rinsed in distilled water, dehydrated through graded series of alcohols, cleared in xylene and mounted with DPX.

To verify the specificity of the antisera used in these studies, control procedures were adopted. They included ommission of one step of the reaction, replacing antisera with normal goat serum and/or absorbing 1 ml of the dilution of each antisera with respective antigen for 24 hours at 4° prior to incubation.

Observation

Present study clearly demonstrated immunoreactivity of the pseudobranchial neurosecretory cells with neurophysin, serotonin and neuropeptide Y, while no immunoreactivity was observed with β endorphin, in both the fish species namely *Clarias batrachus* and *Heteropneustes fossilis* (Fig. 1 & 2) However, stronger reaction in the neurosecretory cells of the pseudobranchial neurosecretory cell was observed with neurophysin (Fig. 1c & 2c) in comparison to serotonin and neuropeptide (Figs. 1a,b & 2a,b) Immunoreactivity was observed in cell soma and cell processes (c.pr) both (Figs. 1d & 2d), suggesting probable active transportation of secretory products to neurohaemal contact sites through these cell processes. It is

interesting to note that all the cells in a single cell mass showed immunoreactivity with the three antisera used, suggesting coexistence of more than one bioactive substance, associated with this neurosecretory system.

All controls yielded negative results.

Discussion

The present investigation demonstrates for the first time the presence of neurophysin, neuropeptide Y and serotonin in the pseudobranchial neurosecretory cells of two Indian catfish species, *Clarias batrachus* and *Heteropneustes fossilis* . Immunohistochemical techniques have been applied for study of neuroepithelial cells (NE cells) in the airways of many vertebrates The first report on identification of amine in NE cells of fish gill filament was provided by Dunel-Erb and coworkers[28] using conventional histoflourescence method. Subsequent immunohistochemical investigations have confirmed co-localisation of serotonin with endothelin peptides and neuropeptides and also that of n NOS with endothelin in the neuroendocrine cells of several fish species, including Indian catfish *Heteropneustes fossilis* and a clupeid *Notopterus chitala*.[20,29] These cells were identified immunohistochemically using antisera against many bioactive substances but among these serotonin was found to be most prevalent. The presence of serotonin or other biogenic amines in neuroendocrine cells that produce polypeptide hormones is quite common.[30-31] These amines are supposedly involved in the synthesis, storage and/or release mechnisms of polypeptide hormones.[37]

Positive immunoreactivity with serotonin suggests a role for the pseudobranchial neurosecretory cells in the respiratory physiology of fishes, as the cells producing serotonin are observed to have a role to play in hypoxia and the effects of serotonin in ventilation have been suggested.[28] Serotonin has been reported to have strong vasomotor effects on fish gills.[31] The neuroepithelial bodies in rabbits, have been found to release serotonin upon exposure to hypoxia.[33-35] The release of neurosecretory products from pseudobranchial neurosecretory cells in response to experimentally provoked hypoxia has been reported in *C. batrachus* and *H. fossilis*[23,36] which ultimately induces surfacing behaviour of these fishes. Although direct evidence for this is still not available, this amine, seems to be functioning as a mediator for hypoxia induced surfacing behaviour. Biogenic amines, including serotonin, have been observed to be colocalized with peptide hormones within neurosecretory granules of APUD type cells, suggesting that the function of these amines may be related to the synthesis, storage and/or release of peptide hormones.[37]

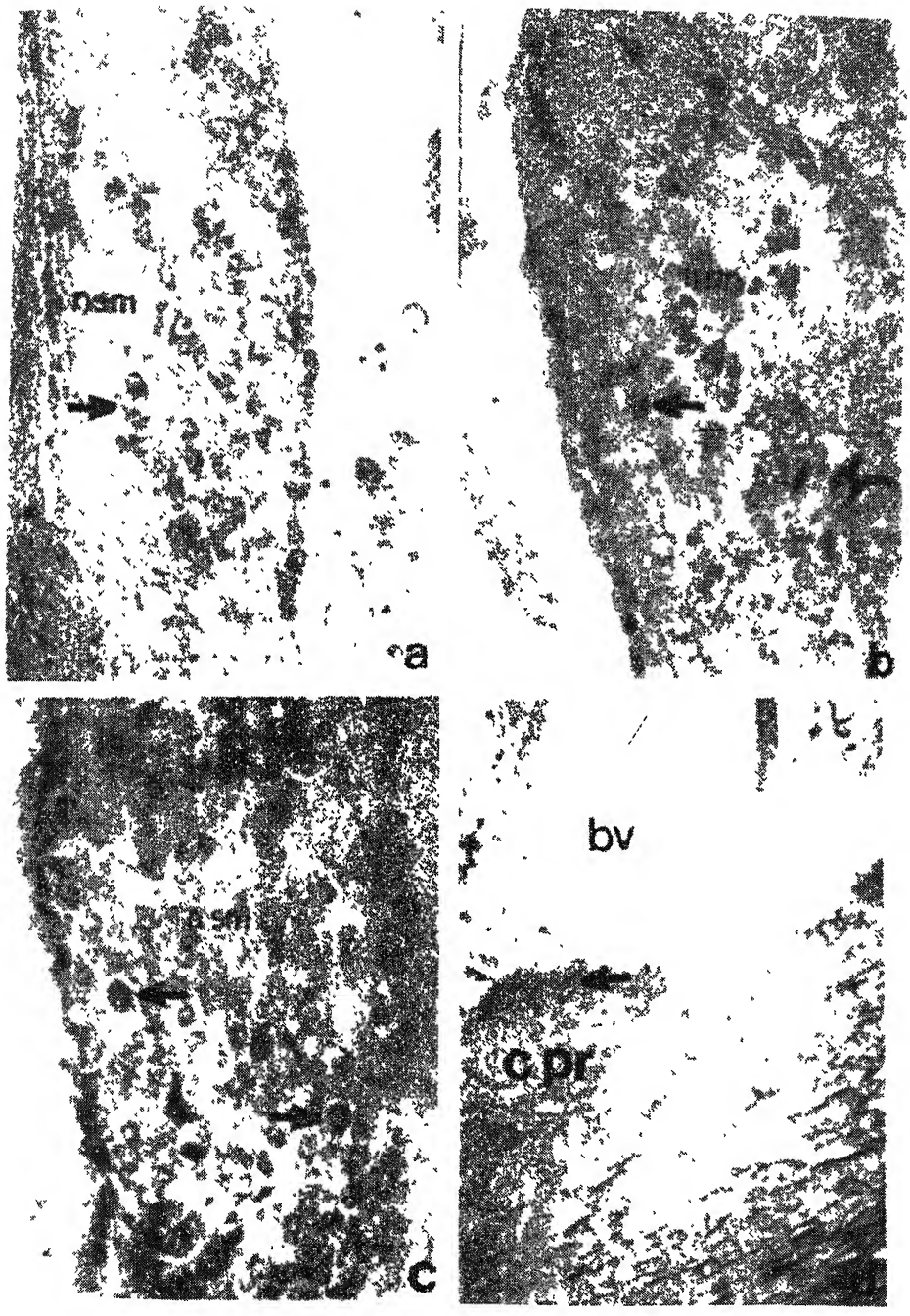

Fig 1– Photomicrographs of Pseudobranchial neurosecretory cells in *Clarias batrachus*, showing positive immunoreactivity

(a) With Neuropeptide-Y – 280 X (b) With Serotonin –280 X
(c) With Neurophysin – 280 X (d) Immunoreactivity of cell processes with neurophysin, seen in neurohaemal contact site between blood vessal (bv) and bundle of cell process (C pr) – 300 X

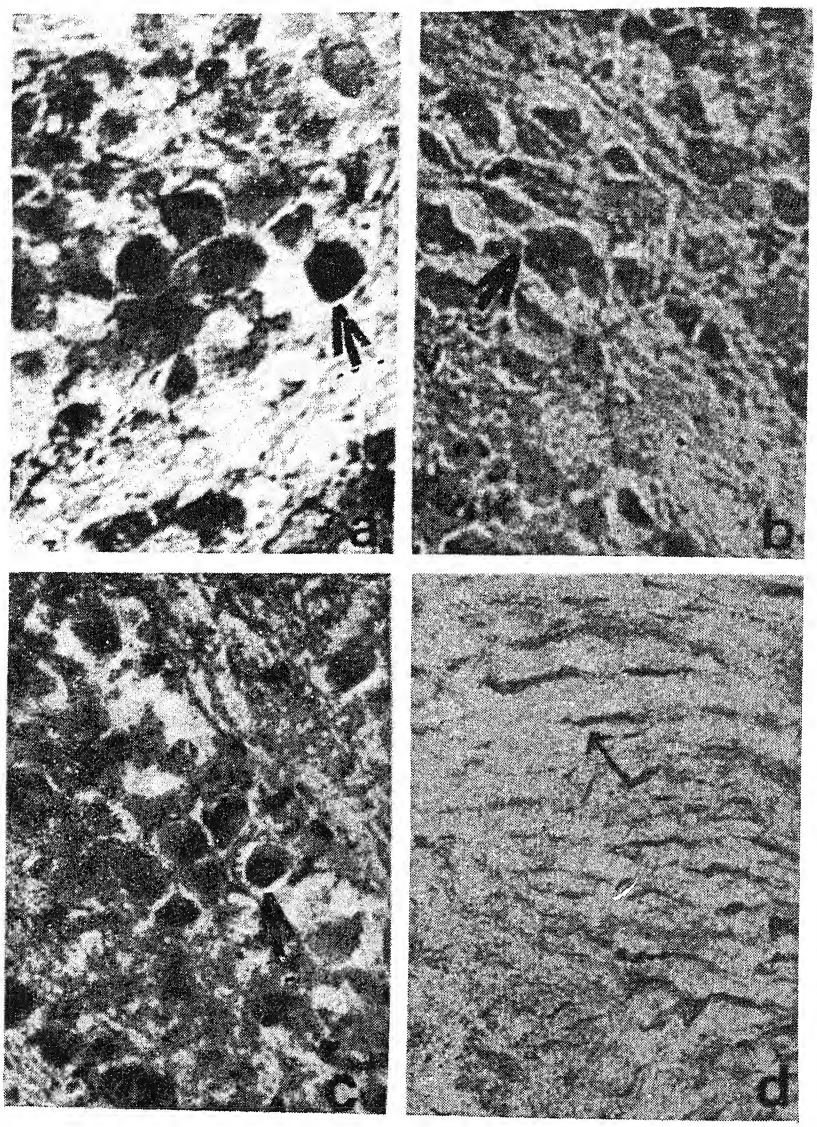

Fig. 2– Photomicrographs of Pseudobranchial neurosecretory cells in *Heteropneustes fossilis*, showing positive immunoreactivity.

(a) With Neuropeptide-Y – 200 X (b) With Serotonin –200 X (c) With Neurophysin – 200 X (d) Immunoreactivity with neuropeptide-Y observed in cell processes (arrows) –200 X

The immunoreactivity with neuropeptide Y- a potent stimulator of food intake, has many significant implication for this system. This observation suggests the production of more than one bioactive principle by the pseudobranchial neurosecretory cells and thus reveals association of this system with more than one function in the biology of these fishes, as neuropeptide Y has been found to be associated with various brain functions like feeding, sleeping, circadian rhythms, etc It is known to participate in the hypothalamic neuroendocrine feed back control of steroid hormone levels in mammals[27,38] and fish[39] and plays an important role in feeding behaviour as well [29-31,40,42] This observation suggests that among other functions, neuropeptide Y may have a role in neuroendcrine regulation of feeding and/or circadian rhythms demonstrated by these fishes

The present investigation throws light on the possibility of this system having a paracrine role in the biology of these fishes It may be presumed that the active principles produced by the pseudobranchial neurosecretory cells are released by exocytosis in the surrounding tissue, major part of which comprises of blood capillaries. This release activates the fish to go to the surface and feed Exocytosis and degranulation are mediated by environmental hypoxia, an effect observed in fish and mammalian lung neuroendocrine cells[28,34,42 43] and also in the pseudobranchial neurosecretory cells.[23,35] Catfishes are nocturnal in habit and are known to show frequent surfacing and feeding activity in the night. It seems possible that serotonin and neuropeptide Y act as mediators for the onset of surfacing and feeding in these fishes.

The presence of more than one bioactive substances in these cells suggests multiple functional roles of these cells in the biology of these economically important group of fishes. More in depth investigation are needed to confirm this.

Acknowledgement

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Growth and development of angoumois grain moth, *Sitotroga cerealella* Olivier on different rice varieties

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Abstract

The growth and development of angumois grain moth, *Sitotroga cerealella* Olivier on 18 rice varieties have been studied under controlled laboratory conditions (Relative Humidity. 75 ± 5% & Temperature 27 ± 2 °C) The results on different growth and development processes, viz fecundity, hatching, pupation, adult emergence, developmental periods, number of generations, adult longevity, F_1 progeny and index of suitability indicated the varieties Sona and T -26 as preferred and the varieties Pant-4 and IR-8 as less preferred host for S *cerealella*

(**Keywords** rice varieties/growth and development/angoumois grain moth/ *Sitotroga cerealella)*

Introduction

Rice suffers post harvest grain loss up to 20 per cent due to various insect pests[1]. Among the stored grain pests, angoumois grain moth, *S. cerealella* Olivier is the most destructive pest causing considerable loss to rice and maize under storage as well as on mature field crops[2,3] Several workers have done researches on different aspects of growth and development of S. *cerealella*[4, 5,6,7]. In view of above, a comprehensive study on the effect of different rice varieties on various aspects of growth and development of *Sitotroga cerealella* has been conducted during the present investigation.

Materials and Methods

The growth and development of angoumois grain moth, *Sitotroga cerealella* Olivier on different rice varieties under controlled conditions at 75±5%relative

humidity and 27 ± 2 °C temperature at Chandra Shekhar Azad University of Agriculture & Technology, Kanpur and Saket Degree College, Faizabad were studied. Eighteen rice varieties viz Pant-4, Pusa-615, T-3, Basmati-370, Sarjoo-52, IR-24, Saket-4, IR-36, Ashwani, Sita, IR-8, T -9, T -26, Prasad, Usar-l, Cauvery, Sona and T -23 have been evaluated as food of the moth.

For determination of the fecundity, one pair of the moth was introduced into specimen tube having 25 gm grains/variety and replicated 3 times Each tube was covered with perforated polythene and tied with rubber band to prevent escape of moth. The incubation period and hatching percentage were recorded. To study the developmental period, 100 newly hatched larvae were kept in specimen jar containing 100 gm grain/ variety and replicated 3 times. To determine the Fl progeny, 5 pairs of newly emerged moths were released into 500 gm capacity jar containing 100 gm grain/variety of 3 replicates. The jars were covered with muslin cloth and tied with rubber band. To find out generations, one pair of male and female moths was released into specimen tube containing 25 gm grain/variety. Deposited eggs were counted daily and recorded the total duration from egg laying to adult emergence for the first generation. One pair of male and female moth was again isolated from the lot of first generation and placed in separate specimen tube. This process was continued throughout the years to determine the total numbers of generations in each variety.

The Index of suitability of rice varieties was calculated by using following formula.[8]

$$I = \frac{\text{Log}_e F}{D} \times 100$$

Where, I = Index of suitability

F = Total numbers of F_1 adult

D = Developmental period in days

The data have been subjected to analysis of variance. The data on fecundity and F_1 progeny were subjected to log transformation and the percentage data to angular transformation.

Results and Discussion

Varietal differences in fecundity, hatching, pupation and adult emergence of *S. cerealella* :

The results on the effect of different varieties on fecundity, hatching, pupation and adult emergence of *S. cerealella* are summarized in Table 1. Maximum number of eggs were laid on variety T-26 (135.271 egg/♀), which were not significantly different from those as in Cauvery, Sona, T-23, Prasad, T-3, IR-8 and Pusa-615 having laid 133.849, 130.103, 126.476, 125.808, 124.674, 123.427 and 123.056 eggs/♀ respectively. However, minimum eggs were deposited on variety Pant-4 (101.121 eggs/♀) followed by Usar-1, Sarjoo-52, IR-36 with 102.213,102.426 and 103.153 eggs/♀ respectively The other rice verities exhibited intermediary fecundity behavior of *S. cerealella.* Similar fecundity behavior have also been observed on different paddy varieties.[6] The hatching percentage was minimum in Pant-4 (73.339 %) and IR-8 (73 339 %) though at par with Usar-1 (74.668 %) and IR-36 (74.668 %). The maximum hatching percentage was recorded in T-26 (89.351 %) and Sona (89.338 %) The minimum pupation was noted in Pant-4 (56.334 %) and IR-8 (56.334 %) followed by IR-36 (59.334 %) and Cauvery (60.334 %). However, the maximum pupation was found in Sona (77.335 %). Minimum pupation on Cauvery (59.08 %) and maximum on Sona (93.76 %) have been reported in past.[7] The maximum adult emergence was observed on varieties Pant-4 (62.334 %) and IR-8 (62.334 %). However, maximum emergence was noted in Sona (82.684 %). Significant differences in the emergence of moth on barley varieties from 26.3 to 52.8 per cent have also been observed by previous workers[5].

Table 1– Effect of different rice varieties on fecundity, hatching, pupation and adult emergence of *Sitotroga cerealella*

Varieties	Fecundity (No./♀)	% Hatching	% Pupation	% Adult emergence
Pant-4	101 121 (2 005)	73.339 (1 028)	56 334 (0 849)	62 334 (0 910)
Pusa-615	123 056 (2 090)	82 669 (1 141)	75 335 (1 051)	80 335 (1 111)
T-3	124 674 (2 096)	82.336 (1 137)	73 339 (1 028)	80 669 (1.116)
Basmati-370	116 505 (2 006)	82 336 (1.137)	73 000 (1 024)	79.669 (1.103)

Table 1 Contd .

Table 1 Contd

Sarjoo-52	102 429 (2 010)	76 000 (1 059)	61 667 (0 903)	65 334 (0 941)
I R-24	116 239 (2 065)	76 005 (1 059)	64 000 (0 927)	72 338 (1,017)
Saket-4	114 502 (2 059)	84 345 (1 164)	69 668 (0 988)	76 335 (1 063)
I R-36	103 153 (2 013)	74 668 (1 043)	59 334 (0 879)	65 334 (0 941)
Ashwani	115 260 (2 062)	77 340 (1 075)	64 667 (0 934)	74 668 (1 043)
Sita	112 926 (2 053)	76 668 (1.067)	63 334 (0 920)	65 336 (0 941)
I R-8	123 421 (2.091)	73 339 (1.028)	56 334 (0 849)	62 334 (0 910)
T-9	115 260 (2 062)	79 335 (1.099)	67 334 (0 962)	72 338 (1 017)
T-26	135 271 (2.131)	89 351 (1 238)	73 668 (1.032)	78 669 (1 091)
Prasad	125.808 (2 100)	82 243 (1 137)	69 337 (0.984)	75 339 (1 051)
Usar-1	102 213 (2 010)	74 668 (1 043)	63 334 (0 920)	68 673 (0 977)
Cauvery	133 890 (2 127)	76 668 (1 067)	60.334 (0 889)	68 003 (0 970)
Sona	130 103 (2 114)	89.368 (1.238)	77 335 (1 075)	82684 (1 142)
T-23	126 476 (2 102)	79 669 (1 103)	66 334 (0 952)	77 679 (1 079)
S Em (±)	0 024	0 009	0 006	0 009
CD (P=0 05)	0.049	0 01 7	0 012	0 018

Varietal differences in developmental period and number of generations of *S. cerealella* :

The result regarding the effect of different varieties on developmental period and number of generations of *S. cerealella* are summarized in Table 2. The maximum developmental period was observed on variety IR-24 (52 days) followed by IR-8 (50.999 days) and Pant-4 (50 days) ; and the minimum on variety Sona (31.334 days). Earlier, it has been reported that the total developmental period was maximum on Saket-4 (39.71 days) and minimum on Ratna (24.42 days).[7] The number of generations varied from 7 to 10 on different rice varieties. The minimum 7 generations were recorded on varieties Pant-4, Sarjoo-52, IR-36, IR-8, Usar-1 and

Cauvery, whereas the maximum on Sona and T-26. Earlier, 7 to 12 generations of *S. cerealella* have been reported on maize varieties[4].

Varietal differences in longevity, sex ratio, Fl progeny and index of suitability of *S. cerealella* :

The results in relation to longevity, sex ratio, Fl progeny and index of suitability of S. *cerealella* are summarized in Table 3. The data on adult longevity indicated the female moth (3.667 to 7.333 days) comparatively lived longer than male moth (3.333 to 5 667 days). Earlier, similar observations have also been made.[9] The minimum male longevity of 3 333 days was observed on varieties Pant-4, Sarjoo-52 and Sita, whereas maximum male longevity was observed on variety Sona (5.667 days). The minimum female longevity of 3.667 days was observed on varieties T-9, Prasad and Cauvery while maximum was on T-26 (7.33 days), which did not differ significantly from Sona (6.667 days) and IR-8 (6.667 days). Variations in the longevity of moths on different rice varieties have also been observed earlier.[6] The sex ratio (female/male) was maximum on variety Sona (1.49), while it was minimum on variety Saket-4 (0.65).

Table 2– Effect of different rice varieties on developmental period and number of generations of *Sitotroga cerealella*

Varieties	Incubation period (days)	Larval period (days)	Pupal period (days)	Total developmental period (days)	No of generation
Pant-4	11 667	30 333	8 000	50 000	7
Pusa-615	6 000	21 333	7 33 3	34 666	9
T-3	5 333	20 333	7 333	32 999	9
Basmati-370	5 667	20 667	7.333	33 667	9
Sarjoo-52	9 667	29 333	8 000	47 000	7
I R-24	9 333	26 667	6 667	52.000	8
Saket-4	11.000	19 667	6.637	37.000	9
I R-36	8 667	28 333	8 000	45.000	7

Table 2 Contd...

Table 2 Contd

Ashwani	5 333	25 333	6 667	37 333	8
Sita	10 667	27 000	8 000	45 667	8
IR-8	11 333	31 333	8 333	50 999	7
T-9	9 667	25 333	7 333	42 333	8
T-26	8 667	19 333	6 333	34 333	10
Prasad	4 667	29 000	6 333	40 000	8
Usar-1	7 667	29 667	7 333	44 667	7
Cauvery	9 333	25 333	7 666	42 333	7
Sona"	5 000	18 667	7 667	31 334	10
T-23	5 333	25 333	7 000	37 666	8
S Em (±)	0 544	0 676	0 535		
CD (P=0 05)	1 104	1 371	1 230		

Table 3– Effect of different rice varieties on adult longevity, sex ratio and F1 progeny of *Sitotroga cerealella*

Varieties	Longevity (days)		Sex ratio (male=1)	F1 progeny (no / ♀)	Index of suitability
	male	female			
Pant-4	3 333	5 667	1 10	51 974 (1 716)	17 90
Pusa-615	4 667	5 000	1 30	70 660 (1 849)	12.28
T-3	4 333	4 667	1.34	73 327 (1 865)	13 02
Basmati-370	3 667	4 333	1.31	73 656 (1.867)	12 76
Sarjoo-52	3.333	5 667	1 24	62 309 (1 794)	8 79
I R-24	4 000	4.333	1 05	66 322 (1 822)	8 07
Saket-4	4 333	5 000	0 65	66 985 (1 826)	11 36
I R-36	3.667	5 333	0 95	54.659 (1.728)	8 89

Table 3 Contd ..

Table 3 Contd

Ashwani	4 000	5 667	1 00	65 980 (1 819)	11 22
Sita	3 333	5 333	1 10	61 978 (1 792)	904
I R-8	3 667	6 667	0 96	47 622 (1 678)	758
T-9	3 667	3 667	0.88	60 930 (1 785)	9 71
T-26	4 667	7 333	1 43	77.983 (1 892)	12 69
Prasad	4 333	3 667	1 28	69 607 (1 843)	10 61
Usar-1	3 667	5 333	0 85	52 255 (1 718)	8 86
Cauvery	4 000	3 667	1 12	57 630 (1 761)	9 58
Sona	5 667	6 667	1 49	78 307 (1 894)	13 92
T-23	4 333	4 000	1 35	58.596 (1 768)	10 81
S Em (±)	0 471	0 430		0 014	
CD (P=0 05)	0 956	0 873		0.028	

In relation to Fl progeny, the highest population of moth was recorded on variety Sona (78.307) and T-26 (77.983) which did not differ significantly. The minimum moth population was observed on variety IR-8 (47.622) followed by Pant-4 (51.974), Usar-l (52.255)and IR-36 (54.659). Higher number of Fl moth on Sona (80.00) and lesser number on T-I00 (15.66) have been reported earlier also[10].

The index of suitabilty of different rice varieties to *S. cerealella* indicated that the highest index of suitability was recorded on variety Sona (13.92). It was followed by T-3 (13.02), Basmati-370 (12.76), T-26 (12.69) and Pusa-615 (12.28). Lower index of suitability were recorded on varieties IR-8 (7.58), Pant-4 (7.90), IR-24 (8.07), Sarjoo-52 (8.79), Cauvery (8.86) and IR-36 (8.89). It has been noted that the varieties with higher index of suitability produced maximum numbers of progeny and vice-versa. Earlier maximum values of index of suitability on varieties Ratna and Sona (14.83 and 14.73) and minimum values on varieties Cauvery and Bala (10.03 and 10.07) have been recorded[7].

It may be concluded that the varieties Sona and T -26 are most suitable and the varieties Pant-4 and IR-8 are less for angoumois grain moth, *Sitotroga cerealella.*

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Effect of antioxidant properties of rhizome of *Zingiber officinale* Rose to prevent peptic ulcer

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Abstract

Oral pretreatment with acetone extract of rhizome with *Zingiber Officinale* Rose of *Zingiberaceae* family, used commonly during cooking, as flavouring agent, at the doses of 150, 175, 200 and 225 mg/kg body weight for 10 days to rats shows significant protective action against indomethacin induced gastric lesions Significant reduction in the level of peroxidised lipid, in terms of thiobarbituric acid reactive species was observed in experimental group, compared to that, of the untreated ulcerated group The specific activities of the in vivo antioxidant enzymes Superoxide Dismutase and Catalase maintained a near normalcy level on pretreatment with the acetone extract, as compared to the relatively lower values in untreated ulcerated group. Indomethacin induced depletion of gastric reduced sulfhydryl (GSH) concentration is also counteracted and maintained almost at a normal level. Oxidative modification on proteins, due to oxidative stress or damage by reactive oxygen species is significantly lowered, and the primary cytoprotective mucus barrier level maintains a comparatively high value The results indicate a significant protective action of acetone extract of *Zingiber officinale* Rose against indomethacin induced gastric lesions and re inforced by an appreciable antioxidant potential, as measured by *in vitro* experiments, the action is presumably governed by free radical scavenging action

(Keywords : ,antioxidant/gastric cytoprotection/*Zingiber officinale* Rose)

Introduction

Zingiber officinale Rose, [Zingiberaceae], is a thick perennial herb with thick tuberous rhizome. Originating, natively from South Asia, it is now extensively cultivated throughout the tropics, including India, China, Jamaica and Japan, Chinese records, dating from the 4th century BC, indicate its widespread use to treat stomach

ache diarrhea, nausea, hemorrhage and rheumatism[1]. The pungent principles are thought to be the most pharmacologically active components of *Zingiber officinale,* including, gingerol, shagaol & zingerone[2,3]. Ginger, has also been shown to be an inhibitor of platelet aggregation. It also inhibits thromboxane formation and pro-aggregatory prostaglandins, apart from causing significant reduction in formation of platelet lipid peroxidation[4]

Conventional medical treatment for peptic ulcer viz, H_2 receptor antagonist, proton pump inhibitors, prostaglandin analogues, are usually, not free from side effects[5,6] accompanied by relatively high cost and consequent non-accessibility to the common people. In Indian traditional medical system, a number of plants and plant products are known to possess potent antiulcerogenic efficiency. A growing body of evidences suggests that, at least a part of the therapeutic values of these plant or plant products may be ascribed to their antioxidant properties of some of them. The treatment of peptic ulcer, initially aimed towards a decrease in acid secretion, has now been diverged towards decreasing the oxidative stress, as in many endoscopically proven ulcer, the rate of acid secretion has remained normal. The role of oxygen derived free radicals in the development of pathogenesis in acute experimental gastric lesions induced by stress, ethanol and non steroid anti inflammatory drugs (NSAIDs) are well known[7,8,9,10], apart from the interactive processes, that involve, an imbalance between the protective and the aggressive factors of gastric mucosa NSAID is associated with the inhibition of cyclooxygenase, COX -II, leading to the inhibition of prostaglandins, and ulcer induction[11]. In fact the underlying mechanism behind induction of peptic ulcer is now, believed to be, injury by free radicals[12].Clinical supplementation with synthetic antioxidant, allopurinol, has been shown to incur increased acceleration of healing process and lesser recurrence of ulcer[13].

Moreover, 75% - 85 % of chronic ulcer in human is due to *Helicobacter pylori* infection in the stomach wall, which potentiates polymorphic nuclear leukocyte oxidative burst, leading to a considerable production of reactive oxygen species (ROS), and overinduction of oxidative stress, which degenerate tissue, causing ulcer[14,15].

Zingiber officinale (or commonly called "ginger"), used in our investigation, is used all throughout the globe during cooking, as a flavouring agent. Some instances of antiulcer activity of the rhizome in ethanol, aspirin and indomethacin model of experimentally developed ulcer, have been reported[16]; 6-gingesulfonic acid monoacyldigalactosylglycerols and gingerglycolipids A,B, and C being some antiulcer principles that have been isolated from it[17].

Although antioxidant property[18] and inhibition of prostaglandin thromboxane, and leukotriene synthesis are some reported pharmacological of *Zingiber officinale,* any evidence of link between antioxidant and antiulcerogenic property is yet to be established. As in cases of plant products which have significant therapeutic values, the synergistic action offered by the extract results in a better protection or healing in pathological conditions than one or more individual active principle, we concentrate in the present work on the acetone extract, rather than the individual components, isolated. This is further supported by the pro-oxidant and antioxidant efficacy being a concentration dependent factor. Here we report the antiulcerogenic effect of acetone extract of rhizome of *Zingiber officinale* and a possible correlation with the antiulcerogenic parameters and *in vitro* and *in vivo* elucidated antioxidant potential.

Materials and Methods

Experimental animals

Charles Foster strain of rats of both sexes, weighing between 120 -150g were used for experiments. They were maintained under alternative light and dark cycle of 12 hr : 12 hr. temperature being controlled at 27 ± 2°C, 78 % relative humidity three per polycarbonate cages. The animals were fed with food pellets (Hindustan Lever India) and were given tap water ad *libitum.*

Chemicals

Research highest purity grade chemicals, were used, for the biochemical estimations, 2-Deoxyribose, thiobarbituric acid, malondialdehyde, SOD, catalase and reduced glutathione were from Sigma (St Louis USA) Chemical Company. Others were either from E Merck, BDH/SRL, India.

Extraction of plant materials

The rhizome part of *Zingiber officinale* was collected from the local market of Kolkata in the month of January -February and the specimen identified and confirmed by Botanical Survey of India. The rhizome, of gross weight of 652g, was mashed in the grinder, and soaked, using acetone as solvent, for 3 days, with intermittent shaking. Normal room temperature was maintained throughout the process which was repeated twice. The pooled aqueous acetonic part was evaporated under vacuum in a rotary evaporator, maintaining a temperature of 40°C and pressure of 1.0 torr, and the

remaining aqueous part lyophilised The dried powder, 15g of dry weight, with a brownish amorphous texture (2.3%w/w) kept in a vacuum dessiccator for further studies.

Preparation of solution

A specific weight as required, of the dried material was macerated with double distilled water in a morter - pestle, using gum acacia as binder, its ultimate concentration being maintained at a constant 2%. It was transferred to a small tube and the volume was made up as required, here in after, referred to as 'Agent' or 'Test substance'.

Animal experiments

Rats were randomly divided into three groups of 6 animals each, as follows : Group A : Normal control, Group B : Ulcerated Control; Group C : Experimental. The animals of group A and B were treated with gum acacia solution, 2%, as vehicle. Those in Group C were fed with the' Agent' at the doses of 150, 175, 200, 225 mg/kg body weight in a suspension of 2 % gum acacia for 10 consecutive days. On the last day of 'Agent' administration, animals of group C and Group B were fasted overnight, and ulcer induction was done using indomethacin ,@ 30 mg/kg body weight, suspended in 2% of gum acacia aqueous solution as solvent. Animals were sacrificed using ether anesthesia, 3 hours after the administration of indomethacin. The antral portion of the stomach were taken for morphological observations, biochemical estimation and measurement of wet weight.

Stomach tissue (antrum) were cut into small pieces and transferred into a glass Teflon homogenizing tube to prepare a 10% homogenate (1g w/v) in phosphate buffer, 2mM pH 7.2, under cold condition. It was centrifuged at 2500 g for 10 min. The supernatant was collected for subsequent biochemical estimations.

Measurement of gastric cytoprotective effect microscopically

The area of mucosal damage was calculated in square millimeter expressed as percentage of the glandular stomach according to Szabo *et. al.*[22] to evaluate optimal effective and/or protective dose of the 'Agent'.

Assay of peroxidised lipid level (TBARS)

Peroxidised lipid content, in terms of malondialdehyde(MDA), was estimated after thiobarbituric acid reactive species (TBARS) method, as described by Dos *et.*

al[23]. Briefly 1 ml of properly homogenized gastric tissue in 2 ml of normal saline was mixed with 24% TCA and centrifuged at 2000rpm for 20 min. To 2 ml of protein free supernatant, 1 ml of fresh TBA (0.67%) reagent was added, mixed thoroughly and heated at 95 0 C for 1 hr on water bath, cooled, centrifuged at 2000rpm for 10 min. The pink chromophore developed was extracted with n-butanol, volume of extraction solvent being kept at a constant 2.0ml. The extracted organic layer was measured at 532nm, using 10μM MDA as standard.

Assay of specific activity of superoxide dismutase (SOD)

The method of Mishra and Fridovich[24] was followed involving inhibition of apinephrine autooxidation in an alkaline medium at 480nm in a VIS-UV spectrophotometer. Readings were noted at 30 sec of interval, and the enzyme activity was measured in arbritary units, considering 50 % inhibition as 1 unit of enzyme activity.

Assay of specific activity of catalase (CAT)

Catalase activity of the stomach tissue was determined according to the method followed by Aebi[25]. 100μl of tissue supernatant was added to a solution containing 3ml of H_2O_2 –phosphate buffer mixture. The change in optical density at 240nm per unit time was a measure of catalase activity. The concentration of the buffer - peroxide, the oxidation medium, was standardized at the above wavelength, to get the standardized value of 0.500±0.010 (d=1.0cm).

Measurement of reduced sulphydryl content

The reduced sulfhydryl content was measured after Ellmann. Briefly, tissue homogenate was taken in 10mM phosphate buffer (pH 8.0) medium and 0.2ml of 10mM DTNB (5.5' dithiobis-2-nitrobenzoic acid) of pH 7.0, and incubated at 37° C for 20 mins and absorbance read at 412nm.

Assay of mucus

The mucus content, a major gastrocytoprotective barrier, in the gastric tissue was estimated as described by Corne *et al*[26] by measuring the amount of alcian blue*, AB*(1% in 3% acetic acid) bound to mucus spectrophotometrically at 615 nm.

Assay of oxidatively modified proteins

Oxidatively modified proteins, a product of free radical injury on *in vivo* native protein, in biological system, was measured in terms of the carbonyl content, according to Lavin *et al*[27] with minor modifications. The nucleic acid was removed prior to protein precipitation, minimizing the nucleic acid -protein interaction, by incubation with 1% streptomycin at 37° C for 5 min , and precipitation after centrifugation at 10,000rpm for 5 min. Protein, precipitated with TCA, was reacted with 2,4 dinitrophenyl hydrazine (DNPH) reagent in acidic condition, for 1 hr, with intermittent shaking at 37°C. The diazotized protein was solubilised in guanidine reagent, adjusting the pH at 2.4 with TFA. Optical density at 240nm was taken and concentration calculated using extinction coefficient of 22.000 cm^{-1} M^{-1}.

Measurement of hydroxyl free radical scavenging action

Hydroxyl radical scavenging action was determined according to the method followed by Halliwell and Gutterige[28]. Briefly, the reaction mixture in a total volume of 1.2 ml contained as follows: potassium phosphate buffer, pH 7.4 (10mM); NaCl (63mM); deoxyribose (0.8mM) with / without plant extractives. The reaction was initiated by adding ferrous ammonium sulphate (21 μM) and the mixture was incubated at 37° C for 15 min. Thiobarbituric acid (TBA), 1% w/w , 1ml was then added, and 1 ml of 2.8% of TCA . The whole mixture was heated at 100°C for 10min, cooled, centrifuged at 1000rpm for 5 mins and the fluorescence measured at 553 nm.

Measurement of *in vitro* inhibition of lipid peroxidation

The method followed was Ko *et al*[29] with minor modifications. Briefly, rat gastric tissue homogenate was freshly prepared from the antral portion of the stomach in 10 vol of Kreb's buffer, pH 7.4. The tissue homogenate was centrifuged at 1000rpm for 20 min and the supernatant was used for lipid peroxidation study. The reaction mixture (1 ml) contained 200 μl of tissue homogenate and 10mM KH_2PO_4 buffer, pH 7.4. Peroxidation was started by adding ferrous ammonium sulphate (20 μM) and 200 μM ascorbic acid in absence or presence of the test agent at different concentrations. The reaction mixture was incubated at 37° C for 30 mins. Absorbance was read spectrophotometrically at 532nm. Butylated hydroxyanisole (BHA) was used as a standard.

Statistical analysis

Analysis of variance (ANOVA) was followed by individual comparison by Students' 't' test for determination of level of significance, among the various groups. '

Results

In this study, we characterized the protection parameters by microscopic assessment and by *in* vivo and *in vitro* antioxidant potency by measuring levels of antioxidants.

Table I reveals the microscopic observations assessing the cytoprotective activity of acetone extract of *Zingiber officinale*. Indomethacin induced ulcerated group of rats showed a number of perforations with blood spots; a thin and flaccid mascular layer was noted in each case. However, pretreatment with the extractive at the doses of 150,175, 200, 225 mg/kg body weight orally for 10 days protected the gastric tissue against lesions induced by indomethacin, as is evident from the significant reduction in ulcer index. From the level of highest reduction in ulcer index, acetone extract of *Zingiber officinale* @ 200mg/kg body weight was shown to offer optimal cytoprotective property.

Table I– Effect of acetone extract of *Zingiber officinale* Rosc on gastric cytoprotection

Treatment	Ulcer index at respective doses[a]				
Doses [mg/kg body weight X 10 days]	0	150	175	200	225
Control, Vehicle	28 5 ± 2 6				
Pretreated with acetone extract		8.2±1 1*	5.2 ± 1 5*	4 3 ± 1 3*	5 9 ± 1 2*

*Significant, compared to the untreated group, $p < 0\ 001$

n/group of rats is 6

[a]Expressed as percentage of the surface of glandular stomach

all values are mean ± SEM

Significant increase in the mucus content, a major gastrocytoprotective barrier, was observed in all the pretreated group of rats compared to the ulcerated control .as shown in Table 2. The levels extrapolated towards the normal value and we found that the group, pretreated with 175mg/kg body weight experienced the highest increase in mucus level.

Table 2– Effect of acetone extract of *Zingiber officinale* Rosc on mucus content in gastric secretion

Treatment	Mucus content -dye bound mg AB[a]
Control, Vehicle	2.1 ± 0 02
Ulcer, untreated	0 98 ± 0 02
Ulcer, pretreated, (mg/kg body weight x 10 days)	
150	1.73 ± 0.02
175	1 81 ± 0 03
200	1 66 ± 0 02
225	1 781 ± 0 03

[a]AB Alcian Blue

values are mean ± SEM

*Significant compared to the untreated ulcerated group, *p< 0.01

n/group of rats =6

We measured the protective effect of acetone extract of *Zingiber officinale* Rosc, against free radical injury on lipids and plasma membrane, as is indicated in Table 3. Peroxidised lipid levels, the ultimate product of ROS injury on lipids, in terms of MDA content, showed significant low values in the pretreated groups, as compared to the ulcerated untreated control. The optimal protection against ROS damage on lipid membranes and moieties is offered at a dose of 200mg/kg body weight, Table 3, also reveals the inducing power of the acetone extract of *Zingiber officinale*, to promote *in vivo* activity of the protective antioxidant enzymes. The SOD and catalase activity

increased significantly after treatment, as compared to the ulcerated untreated group, the specific activity level of the group pretreated with a dose of 200mg/kg body weight was found to be close to the normal control group, in case of both the antioxidant enzymes. A significant increase ($p< 0.05$) in the level of reduced thiol (-SH group) content, a significant marker of antioxidant status of the body, was observed in the pretreated group, as compared to the untreated ulcerated group, as is evident from Table 3.

Table 3– Effect of acetone extract of *Zingiber officinale* Rosc on lipid peroxidation (MDA), superoxide dismutase (SOD), catalase and total sulfhydryl (thiol) group in gastric mucosa

Treatment		MDA level nmole/mg of gastric tissue	SOD units/mg of protein in specific activity	Catalase activity u'nits/mg of protein	Total reduced thiol content (nmole/*mg* protein)
Control, vehicle		3 29 ± 0.85"	7 4 ± 1.04*	12.5 ± 0 258*	98 56 ± 2 1*
Ulcer, untreated		7 69 ± 1.64	4 59 ± 0 8	7 35 ± 1 1	83 59 ± 2 23
Ulcer, pretreated With "Agent" mg/kg body weight X 10 days	150	5 3 ± 0 998*	8 26 ± 1 5*	10 2 ± 1 12	93.1 ± 1 98*
	175	4.4 ± 0 1**	8 11 ± 1 7*	11 3 ± 0 98*	96 17 ± 2 11*
	200	3 6 ± 0 26***	8 41 ± 2 1*	12 15 ± 1 15*	98 52 ± 1 97*
	225	4 42 ± 0.15*	6 25 ± 8 8*	9.10 ± 1 15**	83 99 ± 2 5

Values are mean ± SEM, n/group = 6, for each group -significant, compared to ulcer, untreated, $p<0$ 05 - Significant, compared to ulcer, untreated, $p<0$ 02 - Significant, compared to ulcer, untreated group, $p <0.01$

The status of damage by the oxidative stress on native proteins after treatment with acetone extract of the rhizome is revealed in Table 4. The results show the damaged protein level to be significantly lower in the treated groups, compared to in the untreated, ulcerated group.

Table 4– Effect of acetone extract of *Zingiber* officinale Rosc on oxidatively modified protein of gastric mucosa

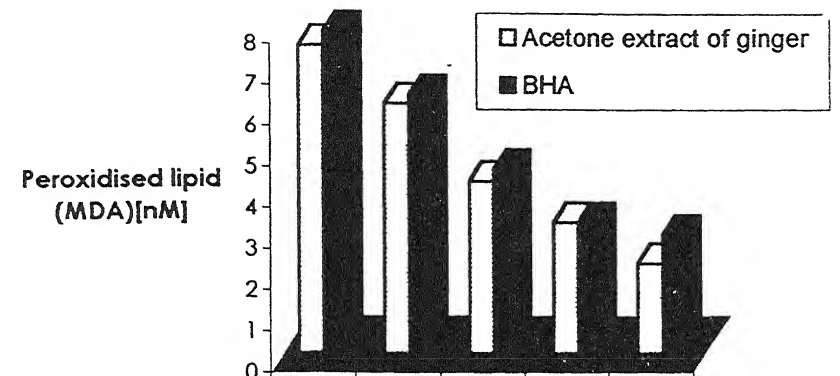
Treatment		Carbonyl content (m) Mean 1. SEM
Control, vehicle		2 1 ± 0 18
Ulcer, untreated		6 1 ± 0 31
Ulcer, pretreated with "Agent" (mg/kg body weight x 10 days)	150	3 15 ± 0 41*
	175	2 66 ± 0 21*
	200	2 5 ± 0 18*
	225	1 9 ± 0 25*

*Significant, compared to ulcerated control, p<0 0l Values are mean ± SEM, n/group of rats =6, In each group

The antioxidant potency of acetone extract of *Zingiber officinale* was confirmed by *in vitro* scavenging action of hydroxyl free radical, as revealed in Table 5. The percentage of inhibition to Fe (II) catalysed oxidation by the acetone extract of the rhizome, showed almost comparable inhibiting power, as of standard synthetic antioxidants. The *in vitro* inhibition of lipid peroxidation by the acetone extract, is shown, as a function of concentration, with relation to the synthetic antioxidant butylated hydroxyanisol (BHA) as shown in Fig. I. The values confirmed a dose dependent inhibition of lipid peroxidation, within the experimental range, adding to the antioxidant power of the acetone extract of rhizome of *Zingiber officinale* .

Table 5– Efficiency of extract of *Zingiber officinale* Rosc to inhibit degradation of deoxyribose

Inhibitor added	% Inhibition of deoxyribose degradation
None	0
Mannitol (10mM)	55
Thiourea (0.5mM)	53
Vitamin C(2mM)	38
Acetone extract (1.0 mg/ml)	37



	1	2	3	4	5
Acetone extract of ginger	7.5	6.1	4.2	3.2	2.2
BHA	7.85	6.32	4.5	3.2	2.9

Concentration [microM BHA/mg per ml of acetone extract]

Fig I–Effect of acetone of *Zingiber officinale* Rose and BHA on *in vitro* peroxidation of gastric membrane lipid

Discussion

The results indicate that treatment with acetone extract of *Zingiber officinale* protects the gastric mucosa from injury by NSAID, viz. indomethacin. The analysis of various parameters, linking peptic ulcer and radical scavenging effects, indicates, that the mechanism of cytoprotective action, to be predominantly antioxidation. The peroxidised lipids are the major end product of free radical injury on lipid membranes, and the noticeable reduction in lipid peroxidation level signifies that the acetone extract scavenges the free radicals before incurrence of damage in the biological system. Thus the acetone extract acts as a chain breaking or sacrificial antioxidant. The reduced level of oxidatively modified proteins, as is evident from the decreased carbonyl content in the experimental group also adds to the evidence ,of the acetone extract acting as a natural antioxidant.

The reduced sulfhydryl content is a significant marker of the antioxidant status of the body. Perturbation of the thiol balance results in serious consequences[30], and the induction of ulcer by indomethacin is associated in the marked reduction of the thiol content, in the reduced state, as is evident from the untreated ulcerated group. In absence of any supplemented oxygen scavenging agent, the oxidative stress is reduced, at the expense of the reduced -SH group. But supplementation with any antioxidant, results in the scavenging of free radicals by the agent, and the level of reduced sulfhydryl group, being kept at the almost normal level. This presumably has occurred after treatment with the acetone extract of rhizome of *Zingiber officinale* Rosc. The supply of natural antioxidant enhance the biological ability to cope with a generalized increase in tissue oxidative stress.

The classical gastrocytoprotective agent mucin is kept at almost a normal value in experimental group, signifying the lower incidence of ulcer and increased cytopotection after pretreatment with the acetone extract of *Zingiber officinale* Rosc. That. not only lowering of ulcer induction, has been shown by indirect parameters, but direct decrease of ulcer index and increase in mucus level confirm the efficacy of the acetone extract to protect NSAID induced experimental ulcer. Morphological observations indicate, a significant reduction in ulcer index, in experimentally induced gastric lesions from indomethacin, in the group pretreated with the extract, (Table 1). The high turnover rate of mucin, a high molecular weight glycoprotein, by its viscoelastic properties and adherence to the epithelial surface, forms a protective barrier. The action can be explained in light of the hydroxyl free radical scavenging action of mucin as mucin is rich in sugar moieties which are potent reducing agents or antioxidants.

Oxygen handling cells have different systems, ego superoxide dismutase (SOD) and catalase (CAT), which are able to protect against the toxic effect of oxygen derived free radicals. We carried out the *in* vitro analysis, to evaluate the hydroxyl radical activity of the extract, in view of the fact, that hydroxyl radical is the most powerful of the damaging ROS. In fact superoxide anion, formed by vasodilation, phagocytosis and fibroblast proliferation reacts with H_2O_2 to form hydroxyl radical, which causes extensive cellular damage by secondary production of peroxyl radical, which is relatively long lived. Superoxide is inactivated by SOD; however, the later is only effective when it is followed up by an increase in catalase or glutathione peroxidase activity, since SOD generates H_2O_2, which is more tissue toxic than oxygen radicals and has to be scavenged by catalase (CAT) or GSH. Thus a concomitant increase in catalase (CAT) or glutathione peroxidase activity is essential,

if a beneficial effect from increased SOD is to be expected. This is exactly what has happened in our experiments, where we find simultaneous increase in SOD and catalase (CAT) levels.

Recent investigational outcomes leading to the role of antioxidants in the healing and reduced recurrences of peptic ulcer provoked us with an attempt to use a natural antioxidant like *Zingiber officinale* as a prophylactic treatment against NSAID induced experimental ulcer. The results indicate a shift of equilibrium of pro-oxidant /antioxidant balance towards the antioxidant side and preventing the unfavourable imbalance of the interactive process of aggressive and defensive factors of gastric mucosa, NSAID induce ulcer by prostaglandin stynthetase inhibition[31,32], through cyclo-oxygenase pathway, and overproduction of leukotrienes and other 5-lipo-oxygenase activity. This in turn inhibits the release of mucus[33]. Though it is not possible to altogether exclude cytoprotective effect exerted by the drug itself, development of ulcer also increases the stomach wall, by increasing the mucus secretion, which would involve triggering other gastric mechanisms in action, including antioxidant activity, which might scavenge as well free radicals[34,35].

Antioxidant treatment, to heal peptic ulcer, or to prevent the oxidative stress, are being exponentially prescribed by physicians nowadays and supplementation with allopurinol along with cimetidine has been documented to heal ulcer at a significantly faster rate than cimetidine alone. The significant toxicity of many a synthetic antioxidants[36,37,38,39] places natural antioxidant as relatively safer in treatment peptic ulcer.

The present study indicates that acetone extract of *Zingiber officinal* provides significant protection against indomethacin induced gastric ulcer in experimental model, and the mechanism is predominantly, a radical scavenging one, Fig. I and Table 5 shows that the acetone extract of rhizome of *Zingiber officinale* Rosc acts as free radical scavenging agent. The objective behind *in vitro* analysis of antioxidant power is that the acetone extract may act as an *in vivo* antioxidant by indirect simulation of the antioxidant status, while, in *in vitro* system, it may not be a radical scavenging agent. But, we find no evidence for this assumption, as in *in vitro* system, the extract acts as an antioxidant, presumably because of the presence of antioxidant principles, already isolated from the rhizome. Thus, we can inter, that this rhizome, which we usually use as a flavouring enhancer, has potent protective action against indomethacin induced peptic ulcer.

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Influence of brassinosteroids on growth, yield, metabolite content and enzyme activities of tomato (*Lycopersicon esculentum* Mill.)

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Abstract

Effect of 24-epibrassinolide and 28-homobrassinolide on growth, yield, metabolite contents and activities of three oxidizing enzymes and one hydrolyzing enzyme of tomato was studied Brassinosteroid - application stimulated the growth and substantially increased the yield of tomato plants The growth promotion was associated with elevated levels of nucleic acids, soluble proteins, reducing sugars, non-reducing sugars and starch Brassinosteroid-treatment enhanced the catalase activity and lowered the peroxidase, IAA oxidase and RNase activities

(**Keywords** brassinosteroids/ tomato/ growth/metabolites/ enzymes)

Introduction

Brassinosteroids are a new group of phytohormones with significant growth promoting nature[1,2]. Brassinosteroids are unique among plant growth hormones by virtue of their close structural similarities to mammalian and insect steroid hormones[3]. Based on the fact that brassinosteroids are reported in all the plants so far tested (9 monocots, 28 dicots, 5 gymnosperms, one pteridophyte and one alga), Sasse[4] suggested that brassinosteroids are probably ubiquitous in plant kingdom. The work with brassinosteroid biosynthetic mutants of *Arabidopsis thaliana*[5], *Pisum sativum*[6] and *Lycopersicon esculentum*[7] have provided compelling evidence that they are essential for plant growth and development[8] The growth inhibition of *Lepidium sativum* by brassinozole, the specific inhibitor of brassinolide synthesis, was found reverted by the exogenous application of brassinolide indicating the necessity of brassinosteroids for plant growth[9]. In the present study the influence of exogenously applied brassinosteroids on growth, yield, metabolite contents and activities of certain enzymes of tomato has been investigated.

Materials and Methods

28-homobrassinolide and 24-epibrassinolide were purchased from M/s. Beak Technologies Inc., Brampton, Ontario, Canada. Seeds of tomato *[Lycopersicon esculentum* Mill var Pusa Early Dwarf (P.E.D)] were obtained from National Seeds Corporation, Hyderabad, India.

The seeds were sown in nursery beds. Twenty-day-old plants were transplanted to earthen pots containing 10 kg of garden soil and compost in 10: 1 ratio. Plants were grown in a glass house under natural day length. Brassinosteroids were supplied to the plants as foliar spray at 3 different concentration levels *viz.*, 0.5 μM, 1.0 μM and 3.0 μM on 35^{th} , 45^{th} and 55^{th} day of sowing. Growth parameters were recorded on 60^{th} day. The leaf material was homogenized using 70% (v/v) ethanol on 60^{th} day and stored in deep freezer for the estimation of biochemical parameters. The enzyme studies were conducted on 70^{th} day. All the fruits which were in different stages of development from each plant were harvested on 110^{th} day and the number of fruits per plant and total weight of all the fruits per plant were recorded.

Soluble proteins: Soluble proteins in the ethanol homogenate were precipitated by adding 20% (w/v) trichloroacetic acid and the precipitate was dissolved in 1% (w/v) sodium hydroxide in whose suitable aliquot protein was estimated following the method of Lowry *et al*[10].

Nucleic acids: DNA and RNA in the alcohol homogenate were separated by Ogur and Rosen[11] method. DNA was estimated using Burton[12] method and RNA estimated by the procedure of Schneider[13].

Carbohydrates: The alcohol homogenate was heated and centrifuged. The supernatant was used for the estimation of total sugars[14] and reducing sugars[15]. Non-reducing sugars were calculated by the formula given by Loomis and Shull[16]. The residue was used for the estimation of starch[17].

For enzyme studies, the leaves were harvested in the early hours of morning and washed with distilled water. The surface water was blotted off and the leaves were kept in an icebox and used for the extraction and assay of the enzymes.

Catalase and Peroxidase : The leaf material was homogenized in chilled phosphate buffer (pH=7). The homogenate was filtered and used for assaying catalase and peroxidase activity. Catalase activity was assayed by Barber[18] method. The reaction mixture contained enzyme extract, hydrogen peroxide and phosphate buffer (pH=7). The reaction was stopped by adding conc. sulphuric acid and the residual hydrogen peroxide was titrated with potassium permanganate.

Peroxidase activity was assayed by employing the method of Kar and Mishra[19]. The assay mixture for peroxidase activity contained phosphate buffer (pH=7), pyrogallol, hydrogen peroxide and enzyme extract. After incubation, the reaction was stopped by adding concentrated sulphuric acid. The amount of purpurogallin formed was estimated by measuring the absorbance at 420 nm.

IAA. oxidase . For IAA oxidase, leaves were extracted by the method of Hillman and Galaston[20]. Leaf was homogenized in chilled phosphate buffer (pH=6.1). The assay mixture contained IAA, enzyme extract and phosphate buffer. The reaction was terminated by adding 10% (w/v) trichloroacetic acid. The residual IAA was estimated by Salper reagent and quantified using IAA standard graph.

RNase The leaf material was ground with potassium acetate buffer (pH=6.5) and centrifuged The supernatant was used for assaying the RNase activity employing the procedure of Corblishley *et al*[21].

Results and Discussion

The foliar application of brassinosteroids resulted in substantial increase in the growth of tomato plants as reflected in increases in the fresh and dry weights of the root and shoot systems (Table 1) Among the two brassinosteroids employed, 28-homobrassinolide was found to be the most effective in stimulating plant growth. Earlier studies conducted with brassinosteriod-deficient tomato mutants provided convincing evidence for the brassinosteroids to be essential for plant growth and development[22].

Exogenous application of brassinosteroids to tomato plants also resulted in increase in the yield of the plants (Table 1). This is in accord with the observation of Kamuro and Takatsuto[23] for the promotive effect of brassinolide in winter season on fruit setting and thickening. The present study revealed the ability of two brassinosteroids in improving the yield of tomato plants.

The growth promotion by brassinosteroids was associated with enhanced levels of DNA and RNA (Table 2) and lowered RNase activity (Table 3). Phytohormones influence the growth by regulating nucleic acid synthesis[24]. The results obtained in the present study with brassinosteroids are in confirmity with the observations made by Key[24] with regard to the then known phytohormones. The increase in the levels of nucleic acids might be due to enhanced synthesis and reduced degradation. The mung bean seedlings, when treated with epibrassinolide, are reported to exhibit elevated activity of RNA polymerase and lowered activity of RNase and DNase[25].

Table 1– Effect of brassinosteroids on the growth and yield (110th day) of tomato plants

Parameters	Brassinosteroid treatment						
	Control	28-Homobrassinolide			24-Epibrassinolide		
		0 5μM	1 0μM	3.0μM	0 5μM	1 0μM	3 0μM
Root fresh wt (g)*	8 23±0.59	14.70±0.76	16.73±0 64	18 16±0 72	11 66±0 72	14 00±0 08	17 00±0 50
Root dry weight (g)*	3 76±0 17	8 40±0 09	8 83±0 09	9 36±0 22	5 43±0.19	6 13±0 09	9 30±0 22
Shoot fresh weight (g)*	14 43±1 83	21.80±0.61	29 96±1 03	30 16±0 72	22 00±1 63	22 33±1 73	25 03±0 21
Shoot dry weight (g)*	6 06±0 31	10 36±0 72	12 16±0 34	13 66±0 19	9 33±0 21	9 93±0 30	10 66±0.19
No. of fruits/plants*	7 00±0 27	14.66±0.27	15.66±0 47	16 00±0 47	12 33±0 54	14 00±0 47	15 00±0 27
Fruit wt (g)/plant*	135.00±4 40	349.00±6 96	373.16±4.28	390 50±2 92	328.16±3 07	353.50±3 68	376 16±4.06

*Mean ± S E.

Table 2– Effect of brassinosteroids on the nucleic acids, soluble proteins and carbohydrate fractions of tomato plants

meters	Control	Brassinosteroid treatment					
		28-Homobrassinolide			24-Epibrassinolide		
		0 5µM	1 0µM	3 0µM	0 5µM	1 0µM	3 0µM
DNA (mg g^{-1} fr wt)*	5.15±0 08	9 12±0 10	10 36±0 19	1 16±0 07	8 3 1±0 15	9.09±0 20	10 12±0 14
RNA(mg g^{-1} fr wt)*	8 42±0.15	10 44±0.44	12 81±0 13	13 57±0 33	10 37±0 36	11 29±0 26	1.75±0 34
Soluble proteins (mg g^{-1} fr wt)*	3 85±0 75	4 99±0 75	5 71±0 21	5 91±0 12	4 80±0 71	5.50±0 21	5 76±0 60
Reducing Sugars (mg g^{-1} fr.wt)*	3 62±0.15	5 92±0 14	6 27±0 09	6 62±0 16	4 35±0 16	4.62±0 24	5 05±0 02
Non-reducing Sugars (mg g^{-1} fr.wt)*	4.58±0. 24	7.12±0 08	7 51±0 18	7.89±0 11	6 59±0 30	7 04±0 06	7 20±0 14
Starch (mg g^{-1} fr wt)*	5.31±0.13	7.57±0.08	8 02±0 11	8 61±0 27	7.05±0 09	7.44±0 11	7.65±0.13

*Mean ± S .E.

The tomato plants sprayed with brassinosteroids showed increased contents of soluble proteins (Table 2). Sasse[26] suggested that brassinosteroids can stimulate the synthesis of particular proteins associated with growth. Supplementing the culture media with 24-epibrassinolide increased cell division rate and amount of soluble proteins in Chinese cabbage protoplasts[27]. The growth promotion in groundnut by brassinosteroids was found to be associated with improved nitrogen fixation[28] and soluble protein content[29]. The alleviating influence of brassinosteroids on salinity stress induced inhibition of growth in rice was found associated with elevated levels of proteins[30].

Foliar application of brassinosteroids caused sharp rise in the levels of all the three carbohydrate fractions in tomato plants (Table 2). The increase might be due to enhanced photosynthetic capacity of the plants as influenced by the brassinosteroids. Infact, increase in CO_2 fixation and levels of reducing sugars was reported in wheat and mustard plants by the application of brassinolide[31] Similarly increase in photosynthesis in wheat treated with 28-homobrassinolide has been reported[32].

The effect of brassinosteroids on the activity of two oxygen scavenging enzymes studied are at contrast (Table 3). The activity of catalase extracted from brassinosteroid treated tomato plants was more as compared to the untreated control plants. On the other hand, the activities of peroxidase enzyme from tomato plants treated with brassinosteroids was less when compared to control plants. Similar reduction of peroxidase activity in 24-epibrassinolide treated hypocotyls of light grown cucumber seedlings[33] and mung bean epicotyls[34] has been reported. The results obtained in case of peroxidase activity of the present study with whole plant system are in confirmity with the earlier observations made with epicotyls and hypocotyls.

IAA-oxidase enzyme extracted from brassinosteroid-treated plants was found to exhibit reduced IAA oxidizing activity (Table 3). Thus, brassinosteroids seems to exhibiting IAA-sparing influence. Similar decrease in IAA oxidase activity in mung bean epicotyls treated with brassinolide was observed[34]. In case of hypocotyls from light grown cucumber seedlings, application of epibrassinolide resulted in lowered IAA oxidase activity. Evidences are in favour that the effect of brassinosteroids on growth is both independent and additive in presence of IAA. The antiauxin, 2(p-chlorophenoxy) isobutyric acid, does not affect brassinosteroid induced elongation indicating that brassinosteroids does not depend on auxin as a mediator in the promotion of younger tissues but probably accelerates auxin effect in mature tissue[26]. On the other hand, the gravitropic curvature in maize primary roots caused by brassinolide was found further promotive in the presence of IAA indicating the interaction between brassinosteroids and auxins[35].

Table 3– Effect of brassinosteroids on the activities of enzymes of tomato plants*

parameters	Control	Brassinosteroid treatment					
		28-Homobrassinolide			24-Epibrassinolide		
		0.5μM	1 0μM	3 0μM	0 5μM	1 0μM	3 0μM
Catalase[a]	32.79±1 41	59.73±1 08	62 53±1 29	68.26±0 81	56 62±1 31	59 24±0 84	61 36±1 01
Peroxidase[b]	0 966±0 21	0 774±0 18	0 761±0 06	0 673±0 04	0 789±0 08	0 777±0 16	0 758±0 12
IAA oxidase[c]	14 33±0 16	13 10±0.26 I	2 80±0 24	2 33±0 16	3 05±0 22	3.01±0 14	2 75±0 28
RNase[d]	0 472±0 10	0.283±0 03	0 240±0 01	0 234±0 03	0 291±0 05	0 275±0 01	0 245±0 04

a : Catalase activity is expressed in terms of enzyme units

b · Peroxidase activity is expressed in absorbance units which indicates the amounts of purpurogallin formed

c · IAA oxidase activity is expressed in terms of amount of IAA destroyed in μg g^{-1} fr wt / 20 min

d : RNase activity is expressed in absorbance units which indicates the amount of nucleotides formed due to depolymerization of RNA

* : Mean ± S E

A great role for brassinosteroids in the 21st century agriculture to increase crop productivity is being predicted[36]. The present study demonstrated the ability of brassinosteroids to enhance the growth and economic yield of tomato.

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Invasion of marble stones by mycoflora

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Abstract

For studying the microbial diversity prevailing on marble stones, the marble pieces were collected from 7 dumping sites in Agra In 40 marble samples 34 species belonging to 12 fungal genera were encountered *Curvularia, Aspergillus, Mucor* & *Fusarium* were the most dominating and frequent genera of fungi commonly present at all the dumping sites *Botrytis, Microsporium, Dematium, Stachybotrys, Chaetomium* were rarely noticeable. Invasion was more conspicuous at the cut edges and grooves present on the marble pieces These samples were poorly spotted and less lusturious in comparison to fresh marble pieces

(Keywords marble stone/mycoflora/dumping sites)

Introduction

Marble is extensively used for buildings and ornamental purposes, crafts and sculptures throughout the world for giving expressions to creative artistic abilities because of its relatively durable nature. The weathering of stone artifacts and monuments has been a matter of great concern for both the conservators and conservation science. The process of stone deterioration has attracted the attention of scientists all over the world. The scientists feel that the whole phenomenon of stone decay can be classified as a physical, chemical and biological[6]. Out of these the biological deterioration is a result of action of different biological agencies like algae, fungi, bacteria, lichen etc. on stone. In fact, the phenomenon of biological deterioration has been found to be quite predominant in particularly in tropical countries where high humidity and temperature are quite conducive for the growth of biological agencies. The present work is an attempt to enumerate the microbes prevailing on marble stones in various locations with different intensity and variability.

Materials and Methods

Marble pieces lying at various dumping sites viz. Sikandra Colony, Kamla Nagar, Dayal Bagh, Trans Jamuna, Shaheed Nagar, Sanjay Place, were collected. These

naturally deteriorated marble samples were collected and brought to laboratory in sterilized polybags. Deteriorated marble samples were subjected for standard microbiological techniques for isolating the microbes inhabiting on them. Standard culture Czapek's dox, PDA, Walker and Calwell media were used, and following methods were used :

- Direct agar transfer method 1 .
- Direct swabbing and serial dilution method2.
- The total population (Total count and percent abundance of microorganisms were calculated by the following formulae.

  a–For marble materials

$$Total\ count/cm2 = \frac{nc \times d}{a \times nr}$$

$$b–\ Percent\ abundance = \frac{T.\ P.\ of\ single\ organism}{T.\ P.\ of\ all\ organisms \times 100}$$

where,

nc = Total no. of colonies

d = -dilution

a = area of marble piece/materials

nr = no. of replicates.

Culture plates were incubated at 28 ± 1 °C for 7 days, identified and maintained on PDA slants.

Results and Discussion

The marble pieces were found to be affected by 34 fungal species belonging to 12 genera (Table 1).

Alternaria sp,. Aspergillus flavus, A niger. Curvularia sp., Mucor hiemalis. Penicillium oxalicum. Fusarium moniliforme were the most frequent fungi found at Shaheed Nagar, Kamla Nagar and Dayalbagh dumping sites. *Acremonium sp., Chaetomium globosum. Trichoderma viride, Gliocladium roseum* were the frequent

fungi at these three sites. Trans Jamuna and Sanjay Place dumping sites showed the high abundance of *Gliocladium roseum* and *Alternaria sp*, while *Phoma sp.* and *Ulocladium roseum* were moderately abundant at these two sites. At Sikandra dumping station, *Penicillium oxalicum* and *Aspergillus flavus* were dominant while *Papulospora sp., Curvularia* and *Botrytis sp.* were present moderately, *Gonobotrys sp* and *Streptothrix sp.* were least abundant at Kamla Nagar, but moderately present at Trans Jamuna and Dayalbagh. *Torula sp* was present at Sikandra and Sanjay Place. *Sirosporium sp* and *Trichoderma viride* were present in less numb. at Shaheed Nagar dumping station. In total there were 20 genera at Kamla Nagar, 26 at Trans Jamuna 32 at Sanjay Place, 24 at Dayalbagh and 20 at Shaheed Nagar and 28 at Sikandra Dumping sites.

Dematium sp, *Geotrichum sp.*, *Nigrospora sp* were rarely present at all sites. *Scolecobasidium sp.* and *Microsporium sp.* were only present at Sanjay Place and Trans Jamuna.

These microbes have the potential to cause plant and animal diseases as well as biodeteriorating of cultural property[3]. *Stachybotrys atra*, *Aspergillus flavus*, *A. niger* and *Penicillium oxalicum* sp. may be considered to be most serious biodeteriogens.

Fungi decompose organic substances and produce harmful acidic metabolites. The various species of fungi which are capable of stone decay produce a variety of organic and inorganic acids and as such, basic rocks are more easily attacked by the fungi. The role or fungi in stone decay has been studied by Strzelczyk[4]. The organic and inorganic acids produced by the fungi solubilize the divalent and trivalent cations by chelation. Significant amounts of Si, Al, Fe, and Mg are freed by the secretion of nitric acid[7]. The release of calcium, iron and aluminum ions is dependent upon the concentration of acid produced by such fungi.

Table 1– Occurrence of Fungi on marble pieces at different dumping sites

Fungi Spe	Sikandra Colony			Saheed Nagar			Sanjay Place		
	No Spc.	Freq	Abu	No Spc.	Freq	Abu	No Spc	Freq.	Abu
Alternaria sp	8	100	23 5	8	100	23.5	5	62 5	14.7
Aspergillus niger	8	100	23.5	8	100	23.5	5	62.5	14 7
A flavus	8	100	23 5	7	87 5	20 5	6	75	17 6
A terreus	7	87 5	20 5	6	75	17.6	5	62 5	14.7

Table 1 Contd

Table 1 Contd

Acremonium sp	4	50	11 76	–	–	–	3	37 5	8 8
Botrytis sp	3	37 5	8 8	6	75	17 6	4	50	11.76
Curvularia sp	5	62 5	14 7	8	100	23 5	8	100	23 5
Chaetomium globosum	5	62 5	14 7	7	87 5	20 5	4	50	11.76
Cladosporium sp	4	50	11 76	4	50	11.76	2	25	5.8
Chrysosporium sp	3	37 5	8 8	–	–	–	1	12 5	2 9
Dematium sp	–	–	–	–	–	–	2	25	5 8
Fusarium moniliforme	8	100	23 5	8	100	23 5	8	100	23.5
Gliocladiun roseum	4	50	11 76	4	50	11.76	3	37 5	8 8
Geotrichum sp	2	25	5 8	–	–	–	1	12.5	2.9
Helminthosporium sp	5	62 5	14 7	–	–	–	4	50	11.76
Hormodendron sp.	3	37.5	8 8	3	37 5	8 8	2	25	5.8
Mucor hiemalis	8	100	23 5	8	100	23 5	7	87 5	20 5
Microsporium sp	–	–	–	–	–	–	–	–	–
Nigrospora sp	2	25	5 8	–	–	–	2	25	5.8
Phoma sp	4	50	11.76	3	37 5	8 8	4	50	11.76
Pithomyces sp	–	–	–	–	–	–	1	12.5	2.9
Populospora sp	4	50	11 76	1	12.5	2.9	1	12.5	2.9
Penicillium oxalicum	8	100	23 5	8	100	23 5	7	87.5	20.5
Rhizoctonia sp				–	–	–	3	37.5	8 8
Sporotrichum sp	2	25	5 8	2	25	6.8	1	12.5	2 9
Scolecobasidium sp	–	–	–	–	–	–	–	–	–

Table 1 Contd ..

Table 1 Contd

Streptothrix sp	2	25	5 8	1	12 5	2 9	1	12 5	2 9
Stachybotrys atra	4	50	11 76	6	75	17 6	3	37 5	8 8
Sirosporium sp	2	25	5 8	1	12 5	2 9	2	25	5.8
Trichoderma viride	4	50	11 76	2	25	5 8	2	25	5 8
Torula sp	2	25	5 8	–	–	–	1	12.5	2.9
Verticicladiella	–	–	–	1	12 5	2 9	1	12 5	2.9
Gonatobotrys	1	12 5	2 9	1	12 5	2.9	2	25	5 8
Ulocladium	1	12 5	2 9	–	–	–	5	62 5	14.7

Fungi Spe.	Trans Jamuna Colony			Kamla Nagar			Dayal Bagh		
	No Spc.	Freq	Abu.	No Spc	Freq	Abu	No Spc	Freq	Abu
Alternaria sp	7	87 5	20 5	8	100	23 5	8	100	23.5
Aspergillus niger	8	100	23 5	7	87.5	20.5	7	87 5	20.5
A flavus	5	62	14.7	4	50	11.76	6	75	17.6
A terreus	6	75	17.6	4	50	11 76	6	75	17.6
Acremonium sp	2	25	5 8	5	62.5	14.7	6	75	17.6
Botrytis sp	–	–	–	5	62.5	14.7	2	25	5.8
Curvularia sp	8	100	23.5	6	75	17.6	8	100	23.5
Chaetomium globosum	4	50	11.76	5	62.5	14 7	4	50	11.76
Cladosporium sp	2	25	5 8	2	25	5 8	5	62 5	14.7
Chrysosporium sp	2	2 5	5.8	1	12 5	2 9	1	12.5	2.9
Dematium sp	–	–	–	–	–	–	–	–	–
Fusarium moniliforme	8	100	23 5	8	100	23 5	8	100	23 5

Table 1 Contd..

Table 1 Contd

Gliocladiun roseum	6	75	17 6	4	50	11 76	5	62.5	14.7
Geotrichum sp	–	–	–				–	–	–
Helminthosporium sp	–	–	–	2	25	5.8	3	37 5	8.8
Hormodendron sp	1	12 5	2 9	–	–	–	2	25	5 8
Mucor hiemalis	6	75	17 6	6	75	17 6	8	100	23 5
Microsporium sp	3	37 5	8 8	–	–	–	–	–	–
Nigrospora sp	–	–	–	–	–	–	1	12 5	2.9
Phoma sp	5	62 5	14 7	7	87.5	20 5	2	25	5 8
Pithomyces sp.	1	12 5	2 9	–	–	–	1	12 5	2 9
Populospora sp	–	–	–	–	–	–	–	–	–
Penicillium oxalicum	4	50	11 76	4	50	11 76	8	100	23 5
Rhizoctonia sp	2	25	5 8	–	–	–	–	–	–
Sporotrichum sp	2	25	5 8	–	–	–	–	–	–
Scolecobasidium sp	1	12 5	2 9	–	–	–	–	–	–
Streptothrix sp	4	50	11.76	1	12.5	2 9	5	62 5	14 7
Stachybotrys atra	2	25	5.8	2	25	5.8	4	50	11 76
Sirosporium sp	1	12.5	2 9	1	12 5	2 9	–	–	–
Trichoderma viride	–	–	–	4	50	11 76	5	62 5	14 7
Torula sp							–	–	–
Verticicladiella	5	62.5	14.7	–	–	–	–	–	–
Gonatobotrys	5	62.5	14.7	1	12.5	2 9	5	62.5	14.7
Ulocladium	4	50	11.76	–	–	–	1	12 5	2 9

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Studies on the aspergilli in the indoor air of some educational institutions at Gorakhpur

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Abstract

This paper presents results of work done on aspergilli of indoor air of classrooms of eleven educational institutions at Gorakhpur The different species of *Aspergillus* were isolated by Gravity Plate method The Petridishes were exposed for 2 minutes at a particular time when the students were present in the class rooms A total of 50 dishes was exposed every month during a period of one year The different species of *Aspergillus* appearing on isolation plates were subcultured and identified.

All the eleven educational institutions were divided into four categories on the basis of rural or urban background of students and hygienic conditions of the classrooms

During this study, between December, 1998 to November, 1999 a total of 17 species of the genus *Aspergillus* were isolated. Ten species were isolated from middle and mixed categories. Six from lower category and four from upper category *A sydowi* was isolated from all the four categories and its percent concentration was highest for the lower category (6) followed by mixed (4 95), upper (0.47) and middle (0.16) Seven species, eg., *A candidus, A. flavipes, A islandicum, A nidulans, A phoenicis, A ustus* and *A versicolor* were present only in anyone of the four categories.

Highest number of species (17) were found in winter followed by rainy (6) and summer (5) seasons. Some of the species appeared only in one season. *A. sydowi* appeared in highest percent concentration in winter (7 03) and in rainy (21.81) seasons

(**Keywords** : *Aspergillus*/air pollution/air-spora).

Introduction

The air near the earth's surface contains a rich population of spores of micro-organisms and pollen. This constitutes the air-spora. The hyphal fragments and spores of air, particularly air borne conidia and ejected ascospores and basidiospores adapted for aerial dispersal constitute the fungal component of the air spora. The aerobiology has been most ably summarised by a few[1,2,3]. Many air borne fungal spores are responsible for biodeterioration of storage materials, equipments, library materials and archives[4,5]. The fungal spores which are present in the house dust, indoor air of

factories, industries or classrooms etc. have been reported to cause various types of allergic reactions in susceptible individuals[6,7]. From the literature available it has been found that the work on air spora is largely related with the outdoor air. Very little work has been reported on indoor air, particularly on the indoor air of class rooms of educational institutions

Materials and Methods

The different species of *Aspergillus* were isolated by exposing the Petridishes of 6.5 cm.diameter containing Martin's peptone dextrose agar medium with rose bengal and streptomycin[8] to the air in the class rooms of eleven educational institutions at different heights The Petridishes were exposed at 2, 3, 4, 5, 5.3 and 5.5 feet above from the floor of class room. The exposure of Petridishes was made for 2 minutes at 11 a.m. when the students were present in the class rooms. Generally one to two doors and 2-4 windows were there in each class-rooms. Most of the classrooms were situated on ground floor but few of them on the first floor. Three Petridishes were exposed at a time. This was done for one year. After exposure the Petridishes were brought back to the laboratory in pre-sterilized polythene bags and incubated at room temperature. A total of 50 dishes was exposed every month during a period of one year. The different species of *Aspergillus* appearing on isolation plates were sub-cultured and identified.

Percent concentration of a species was calculated by dividing the total number of a species isolated from different educational institutions to the total number of all the species isolated from different educational institutions and multiplied by 100.

Results

The eleven educational institutions have been divided into four categories on the following basis :

[1] *Lower Category* : In which most of the students were from rural background and schools surrounded by sparsely populated areas with minimum/poor hygienic conditions.

[2] *Middle Category* : In which most of the students were from semi urban background and schools surrounded by thickly populated areas with better hygienic conditions.

[3] *Upper Category* : In which most of the students were from urban background and schools surrounded by less thickly populated areas with good or best hygienic conditions.

[4] *Mixed Category* : Students from all the above three backgrounds and schools were surrounded by sparsely populated areas with normal hygienic conditions.

Table 1– Percent concentration of *Aspergillus* isolated from indoor air of classrooms of different categories of educational institutions at Gorakhpur.

Fungi isolated	Categories			
	1	2	3	4
1. *Aspergillus candidus*	-	-	-	0.10
2. *Aspergillus clavatus*	.60	-	-	0.21
3. *Aspergillus flavipes*	.90	-	-	-
4 *Aspergillus flavus*	-	.16	-	1.39
5 *Aspergillus humicola*	-	.08	-	-
6 *Aspergillus islandicum*	-	-	-	0.74
7 *Aspergillus koningi*	-	.08	-	.21
8 *Aspergillus nidulans*	-	-	.09	-
9. *Aspergillus niger*	.60	-	1.13	2.56
10 *Aspergillus ochraceous*	.60	.08	-	-
11 *Aspergillus phoenicis*	.15	-	-	-
12 *Aspergillus sachari*	-	.75	-	.10
13. *Aspergillus sydowi*	6.0	.16	.47	4.59
14 *Aspergillus terreus*	-	.16	.28	.32
15. *Aspergillus terricola*	-	0.8	-	.10
16. *Aspergillus ustus*	-	.16	-	-
17. *Aspergillus versicolor*	-	.08	-	-

1 = Lower category;3 = Upper category

2 = Middle category;4 = Mixed category

– = absent.

During studies on indoor air of eleven educational institutions at Gorakhpur district between December 1998 to November 1999 a total of 87 sporing species belonging to 25 genera and 20 sterile mycelial forms were isolated. Out of 87 sporing species 79 belong to Deuteromycotina, 4 to Zygomycotina and 4 to Ascomycotina. In Deuteromycotina group most frequently occurring and dominant species were those of *Aspergillus*.

Out of 17 species of *Aspergillus* 10 were isolated both from category 2 (Middle) and 4 (Mixed), 6 from the category 1 (lower) and 4 from the category 3 (upper). There was only one species i.e., *A. sydowı* which appeared on Petri-dishes exposed to all the four categories. *A. niger* and *A terreus* were isolated from lower, upper, mixed categories and middle, upper and mixed categories respectively. *A. candidus, A. flavipes, A humicola, A. islandicum, A. nidulans, A. phoenicis, A. ustus, A. versicolor* were present only in anyone of the four categories. *A. candidus* and *A. islandicum* in mixed category; *A. flavipes* and *A. phoenicis* from lower category; *A. nidulans*, from upper category; *A. humicola, A. ustus* and *A. versicolor,* from middle category.

Six species were isolated from any two categories *A. clavatus*, *A. flavus, A. koningi, A. ochraceous, A. sacharı* and *A. terricola* were isolated from category 1, 4; 2, 4; 2, 4; 1, 2; 2, 4; 2, 4; respectively.

Percent concentration of *A. sydowi,* which was found in all categories, was higher in lower (6.0) followed by mixed (4.59), upper (.47) and middle (.16). Percent concentration of *A. niger* and *A. terreus* was higher in mixed category. The species which were found in any two categories eg. *A. clavatus* and *A. ochraceous* show higher percent concentration in category 1, *A. sachari* in category 2 and *A. koningi* and *A. terricola* in category 4.

A. humicola, A. koningi, A. ochraceous, A. terricola and *A. versicolor* had lowest percent concentration.

Seasonal pattern of occurrence of different species of *Aspergillus* exhibits that *A. niger, A. sydowi* and *A. terreus* were found in all the three seasons i.e., summer, winter and rainy. The percent concentration of *A. niger, A. sydowi* and *A. terreus* was higher in rainy seasons i.e., 5.43, 21.81 and 10.0 respectively (Table -2).

Highest number of species were found in winter season i.e., 17 followed by rainy (6) and summer (5). *A. candidus, A. flavipes, A. humicola, A. nidulans, A. phoenicis, A. sachari, A. terricola, A. ustus* and *A. versicolor* appeared only in winter season. In

winter and rainy seasons *A. sydowi* was isolated in highest concentration (7.03, 21.81) and *A. nidulans* and *A islandicum* were in lowest concentration i.e., 0.053 and 1.38 respectively.

In summer months *A. niger* was present in highest concentration (3.23) and *A. clavatus, A. koningi* and *A. terreus* in lowest concentration i.e., 0.97.

Table 2– Percent concentration of species of *Aspergillus* in different seasons isolated from indoor air of class rooms of educational institutions at Gorakhpur

	Fungi isolated	Seasons		
		Summer	Winter	Rainy
1	*Aspergillus candid us*	-	1.21	-
2.	*Aspergillus clavatus*	0.97	0.72	-
3	*Aspergillus flavipes*	-	3 61	-
4.	*Aspergillus flavus*	-	0.95	1.81
5.	*Aspergillus humicola*	-	0 08	-
6.	*Aspergillus islandicum*	-	2 11	1 38
7	*Aspergillus koningi*	0.97	0 34	-
8	*Aspergillus nidulans*	-	0.053	-
9.	*Aspergillus niger*	3 23	1.73	5.43
10	*Aspergillus ochraceous*	-	0 97	5.00
11.	*Aspergillus phoenicis*	-	0 60	-
12	*Aspergillus sachari*	-	0.66	-
13	*Aspergillus sydowi*	2 08	7.03	21.81
14	*Aspergillus terreus*	0 97	0.14	10 0
15.	*Aspergillus terricola*	-	0.63	-
16.	*Aspergillus ustus*	-	0.106	-
17.	*Aspergillus versicolor*	-	0.08	-

Discussion

The aspergilli occurring in air have been studied in the present investigation by Petridish exposure method (gravity method) using Martin's Peptone Dextrose agar medium with rose bengal[8], as the isolation medium. The gravity methods provide information on the relative abundance of numerous genera, particularly unspecialised saprophytes and of their numerical fluctuations[9] and on the number and nature of all viable spore and hyphal fragments which will grow on a particular medium. A more precise account regarding identification of spores can be obtained by this method as compared to the direct examination of the spore catch on a sticky slide. If a daily sample is taken under the same conditions, gravity methods give an excellent indication of seasonal changes[10]. It was pointed out[1] that restriction of culture plate method to viable and cultivable particles may be developed with advantage to a high degree of selectivity when sampling is aimed at a limited group of organisms. Since only species of *Aspergillus,* most of which are saprobes, were proposed to be studied, the Petridish exposure method was considered to be particularly suitable for the present study. Martin's Peptone Dextrose agar containing rose bengal and streptomycin has been employed in the present study. This medium was found best for isolating fungi from air in Kansas[11] Such a medium has several advantages when used for the isolation of air borne fungi. The growth of most of the bacteria and actinomycetes is suppressed and the fungal colonies remain small and can be easily counted. Exposure of this medium to sunlight was avoided since photodynamic changes in the dye have been found to suppress colony formation in many fungi[12].

In the present study a total of 17 species of *Aspergillus* have been isolated from indoor air of classrooms of eleven educational institutions at Gorakhpur. Such a large number of species of *Aspergillus* have not been identified previously in the indoor air of any other place by investigators who have tried to study the entire fungal content of the air[13,14]. The present study thus emphasises the need for extensive study of a single genus in the indoor air of classrooms of educational institutions or other such institutions at a time.

The genus *Aspergillus* has been commonly reported, from air in aerobiological studies. Although *Aspergillus* is one of the frequently isolated genera in India[15], but it has not been most frequently reported from the indoor air. There is probably no record about the occurrence of different species which are being reported in this paper from the indoor air of classrooms of educational institutions.

The species which have been reported from the air by the earlier workers are *A candidus, A flavus, A nidulans, A. niger, A sydowi, A terreus* and *A versicolor*[16].

The species which are isolated in all the seasons have been considered to be constant species. The occurrence of such species is based on number of colonies of a species, isolated in different months In this study *A niger A sydowi* and *A. terreus* were isolated during all the three seasons of the sampling periods. Thus these are being reported as constant species.

The percent concentration of various species in the indoor air during 12 months is given in Table-2. The species i e., *A sydowi* and *A terreus* constituted 10 or more than 10 percent of the total isolations.

The lowest number of species i e., 4 were isolated from the upper category (Table-1). These species appeared in lower concentrations. This probably confirms the good or best hygienic conditions of class rooms.

Experiments on the allergic nature of species of *aspergilli* are under observation.

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Growth and chlorophyll content of *Abelmoschus esculentus* as affected by cadmium supplementation

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Abstract

Effect of Cadmium on the growth and physiology of *Abelmoschus esculentus* has been studied Supplementation of Cadmium (200-400mg/kg soil) drastically reduced root length, shoot length and biomass yield It is observed that the initial effect of Cadmium supplementation is reduction in the chlorophyll content ($p<0.01$) which may be due to inhibition of photosynthesis

(**Keywords** growth/chlorophyll/*Abelmoschus esculentus*/cadmium)

Introduction

In recent years research has been focussed on accumulation of heavy metals in crop plants and naturally growing weeds[1] Cadmium is one of the most common toxic heavy metals and has to be prevented from entering the food chain (Haghiri[2]). Widespread contamination of agricultural soils with cadmium has been reported to occur from the heavy use of phosphatic fertilizers as cadmium content of these fertilizers varies from 2 to 200ppm[3]. Cadmium is a potentially toxic metal that can accumulate in the human body with a half-life exceeding ten years[4] Transferring of potentially toxic elements from soil to plants has been well documented[5,6]. The primary point of entry for cadmium into the plants is through the roots; however, for its efficient removal from the soil, it must first be translocated to the harvestable parts of the shoot[7]. Interest in cadmium is also due to its persistence in the environment and its relatively rapid uptake and accumulation by food-chain crops. The cadmium contamination is known to affect the plant regarding seed germination, seedling growth, photosynthesis, respiration, etc. Most of the studies are carried out in human beings and animals and its effects on the plant system are relatively unknown. Hence

the present investigation is carried out to find the phytotoxic effects of cadmium supplementation on *Abelmoschus esculentus.*

Materials and Methods

Investigations were carried out in the research plot of the Allahabad Agricultural Institute-Deemed University in May 2002 to analyze the phytotoxicity caused by cadmium on *Abelmoschus esculentus*

The different concentrations of cadmium used were 0, 200, 300, 400mg/kg soil in the treatment combinations viz. T_0, T_1, T_2 and T_3 respectively. The plant samples were separated into root, stem, leaves and fruits. The samples were washed with tap water and distilled water followed by demineralized water. The soil (sandy loam) used for the experiment was sun dried at room temperature for three days, heat treated and purified by passing through a 2mm sieve in order to eliminate foreign matter and was then filled in pots (25 diameter). Seeds of *Abelmoschus esculentus* were soaked in water overnight, air dried and then sown in pots (5-7 per pot). Frequent irrigation was given to the plants untill they were one month old following which, cadmium supplementation was done. All the experiments were conducted in triplicate to reduce the margin of error. The data presented were recorded on 82DAS. Estimation of root length, shoot length and biomass was done using standard protocols. Chlorophyll content of the leaves was extracted using the method of Hiscox and Israelstam[8] and the chlorophyll estimation was done using Spectronic UV-VIS Spectrophoto-meter.

Results and Discussion

An elevated level of cadmium in plant food creates a potential hazard to human health. Vegetables contribute a higher amount of cadmium to the human diet than foods of animal origin (McKenna and Chaney[9]). Our study has shown that cadmium supplementation of 200-400 mg/kg soil causes adverse effects on root length, shoot length, biomass accumulation and chlorophyll content of *Abelmoschus esculentus* (Table 1). The above mentioned parameters decreased concurrently with the increase in cadmium application from 200 to 400 mg/kg soil indicating that the root length, shoot length, biomass accumulation and chlorophyll content of *Abelmoschus esculentus* showed inverse relation with the cadmium concentration. The reduction in root length at different concentrations of cadmium application was found to be statistically significant. Root water uptake process is inhibited by cadmium, which depends upon the root system architecture (Barcelo and Poschenrieder[10]). The root length of the plants treated with cadmium shows considerable decrease when

compared to control plants and this may be attributed to the fact that the roots are directly exposed to the heavy metal stress and hence show drastic changes. Cadmium is not essential for plant nutrition or metabolism and is probably taken up passively by roots and usually confined to the roots[11]. The shoot length also shows drastic reduction as compared to control plants. The reduction in shoot length is also found to be statistically significant. The shoot length has reduced by 35%, 41 % and 48% with 200, 300 and 400mg cadmium. The reduction in root and shoot length may be attributed to the interference of metal with auxin related cell elongation[12]. The fresh weight and biomass also decreased by 47%, 57% and 60% with 200, 300 and 400mg of cadmium. These results were found to be statistically significant. The total chlorophyll content decreased significantly due to cadmium treatment and 25%, 40% and 45% reduction was noticed with 200, 300 and 400mg of cadmium. The results were found to be statistically significant. Other works having similar results have also been reported Porter and Sherinder[13], Sinha *et al.*[14]. Cadmium has been found to inhibit photosynthesis[15]. The reduction in chlorophyll content may have decreased the rate of photosynthesis and resulted in low biomass accumulation due to cadmium, as has been observed in many plants[16].

Table 1– Root length, shoot length, biomass and chlorophyll content of *Abelmoschus esculentus* in response to cadmium supplementation

	Cadmium supplementation (mg/g FW)			
	0 (To)	200 (T_1, T_2	300 (T_2) and T_3)	400 (T_3)
Root Length (cm)	27 83 (±.52)	17 23 (± 13)	18 46 (±.15)	17 10 (± 23)
Shoot Length (cm)	111 36(± 64)	72 10(±.23)	65 20 (± 12)	57 93 (± 21)
Biomass (g)	64 67 (± 73)	34 15 (± 26)	27 28 (± 21)	25 71 (± 29)
Chlorophyll Content(mg/gFW)	8.77 (± 27)	6.59 (±.19)	5 37 (± 52)	4 81 (±.37)

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Inhibitory effects and allelopathic potential of volatile oils from *Prinsepia utilis* Royle

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Abstract

Allelopathic potential of volatiles released from *Prinsepia utilis* have been studied on wheat and mustard seed germination Volatiles released, strongly inhibited germination in test seeds It is further shown that such inhibition is due to inhibition in respiratory activity in germinating seeds (*TTC* reduction test) GC/MS analysis of volatiles showed linalool and 2-undecanone as major components while 18-20 minor components were also present The inhibitory effect could be due to anyone or combination of these compounds

(**Keywords** volatile oils/*Prinsepia utilis*/allelopathic potential)

Many plants are known to release volatile compounds in air and most of these volatiles come from the class of terpenes. The physiological function of such aroma has been studied in detail mostly with reference to insect attractants / repelants[1]. That such aroma released by any plant also affects other plants growing in vicinity has been less studied.

Eucalyptus trees, releasing volatile oils have been shown to inhibit seed germination and seedling growth in a variety of test plants[2-3]. *E citridora* volatile oils were rich in citronellol where as *E globulus* and *E. tereticornis* volatile oils were rich in cineole, α-pinene and -β-pinene. In nature these volatile oils settled down on soil and inhibited germination and root growth of surface feeders[4]. Some other genera studied, which release volatile compounds are *Artimesia, Salvia, Parthenium* and *Metasequoia*[5-6]. *Prinsepia utilis* (Rosaceae) is a dominant shrub species of lower Himalayan region. Here we show that volatiles released from its leaves have a strong allelopathic potential.

In order to study the effects of volatiles released from *Prinsepia* leaves, such leaves (in gms) were enclosed in a transparent polythene Zip-loc bag (12X14 cm). Higher weight of leaves developed higher levels of volatiles inside the bag. Quantitative levels of volatiles could not be determined and were presumed to be proportional to increase in weight of leaves. Such bags also contained Petri dishes, lined with moistened filter paper and phytometer seeds (sterilized with 1 % $HgCl_2$) for germination counts. A zip-loc bag without leaves was used as control. Such bags were

incubated at 30±2°C and germination counts made at regular intervals. Experiments were performed in duplicate and repeated three times. For TTC reduction studies method of Sharma et al[7] was followed and components of volatiles were identified by GC/MS on Perkin-Elmer, quadrupole MS system.

Effects of volatiles released from *Prinsepia* leaves on seed germination and seedling growth of wheat (*Triticum aestivum* cv. Hs-240) is shown in Table 1. It is clear that increasing levels of volatiles cause increased inhibition of wheat seed germination At 6 gms of leaves after 24 hrs. there was complete inhibition while at later periods of 96 hrs, when control seeds showed 99% germination, treated seeds showed only 30% germination Thus a 70% inhibition is apparent. At lower levels of volatiles similar trend is noticed It is also noticed that with time there is some recovery in germination percentage. This is most probably related to higher vigour of germination shown during 2-4 days of germination. Shoot and root growth was also severely inhibited by volatiles

Table 1– Effect of volatiles from dry leaves of *Prinsepia utilis* on seed germination of *Triticum aestivum* cv. Hs-240

	Germination Percentage ± S D					
Treatment	24 hours	48 hours	72 hours	96 hours	Shoot length* (mm)	Root length* (mm)
Control	80 00±5 09	96 66±1 92	96 66±1 92	98 88±1 92	73 50±2 38	161.17±2 60
2 gms	20.00±3 33	73 33±3 84	83 33±3 33	93 33±3 33	27 85±5 88	38 00±5.75
	(25)	(75 86)	(86 20)	(94 38)		
4 gms	13 33±1.92	60 00±3 33	66 66±3 85	76 66±6 66	12 20±5 44	7 25±1 74
	(16.66)	(62 07)	(68 96)	(77 52)		
6 gms	00 00±0	20 00±1 92	26 66±3 84	30 00±3 33	11 11±4.72	5 90±1 06
	(0)	(20 69)	(27 58)	(30 33)		

* After 96 hrs of growth, data in parenthesis is % of control

Table 2 shows effects of *Prinsepia* volatiles on seed germination of another phytometer i.e., *Brassica napus* cv. sheetal HPN-l. Here again we see that at 6 gms. of leaves there was 100% inhibition of seed germination after 24 hrs. however, after 96 hrs. germination percentage was ca. 40% i.e., 60% inhibition occurred. At lower levels also similar trend was seen. Some recovery in germination % with time seems to be

related to higher vigour of germination during later periods. In order to check the site of action of these volatiles in seed germination, the effect of these volatiles was also checked on the dehydrogenase activity (TTC reduction test) in germinating wheat seeds. Table 3 , clearly shows that increasing levels of volatiles developed inside the Zip-loc bags inhibited TTC reduction by seeds. At 6 gms of leaves when germination was inhibited by 100%, there was ca. 60% inhibition in TTC reduction. Thus the inhibition in seed germination is related to respiratory inhibition caused by volatiles.

Table 2– Effect of volatiles from dry leaves of *Prinsepia utilis* on seed germination of *Brassica napus* var sheetal HPN-1

Treatment	24 hours	48 hours	72 hours	96 hours
Control	33 33±t3 33	88 88±5 09	97 77±1 92	98 88±1 92
2 gms	7.77±1 92	35 33±3 33	65 77±6 67	74 44±5 09
	(23 31)	(39 75)	(67 27)	(75 28)
4 gms	11 10±3 85	25 55±1 92	63 3 3±6 67	41 10±5 09
	(33 30)	(28 74)	(64 77)	(41 56)
6 gms	0.00±0	9 99±3 33	34 44±5 09	39 99±3 33
	(0)	(11 23)	(35 22)	(40 44)

Data in parenthesis represents % of control

Table 3– Effect of volatiles from dry leaves of *Prinsepia utilis* on inhibition of TTC reduction in *Triticum aestivum*

Treatment	Germination %age	µg TTC reduced/g f w./h ± S D
Distilled water (Control)	80	79 93 ± 6.89
2 gms.	20	64 43 ± 5 30
		(19 40)
4 gms	13	41.34 ± 10 83
		(48 28)
6 gms	0	30 94±1 95
		(61 30)

Samples include radicle and the data in parenthesis represent percentage inhibition of TTC reduction

Kohli[8] reported that volatile terpenes present in *Eucalyptus* contribute maximum towards allelopathic potentials. He showed variable response with respect to *Phaseolus aureus* seed germination. Yun[9] reported inhibition of *Achyranthes japonica* seed germination by volatiles released from *Artemesia* plants. Inhibition was concentration dependent. *GC/MS* analyses showed presence of 36-50 compounds from essential oils and bioassays recognized terpene-4-0l, cineole and (-) thujone as potent growth inhibitors. Kong *et al.*[10] reported that fresh leaves and volatiles from *Ageratum conzoides* were inhibitory to seedling growth of various crop plants. Wei-Chun *et al*[6] showed that volatiles from leaves of *Metasequoia glyptostroboides* inhibited seedling growth of wheat and mustard Thus present results with *Prinsepia* confirm earlier reports of inhibition of germination and seedling growth by volatiles.

Regarding the active compounds present in the *Prinsepia* volatiles, GC/MS analysis of steam distillates from *Prinsepia* leaves showed presence of linalool (50.4%) and 2-undecanone (25.7%) as major components and limonene (0.3%), bergamal (0.6%), linalool-oxide-cis (0.4%),sabinene (0.7%), terpineol-trans-β (1.6%), iso-menthol (0.8%), α-terpineol (0.4%), 2-dodecanol (0.4%) and tridecanone (2.0%). Other eight peaks could not be identified clearly[11].

Real active compound in the present study could not be ascertained due to non-availability of pure compounds. The inhibition could be due to anyone or combination of these compounds. Earlier, camphene, camphor, cineole, dipentene, α-pinene, and β-pinene have been identified as volatile inhibitors from several shrub species of southern California[12-14]. Kohli[8] reported that 4 nM/ ml of *Eucalyptus tereticornis* oil inhibited rooting capacity of *Phaseolus vulgaris* hypocotyl cuttings. Citronellol and cineole were reported to be highly effective. Yun[9] found terpene-4-ol, cineole and (-) thujone among 36-50 compounds from *Artimesia* as strong growth inhibitors. Sabinene, terpene-4-ol, 1-8 cineole, linalool, limonene and bergamal present in *Prinsepia* volatile are common with aromatic allelochemicals listed by Mathela from aromatic and medicinal herbs and shrubs[15]. Since dry leaves of *Prinsepia* posses significant aroma which acts as allelochemical, therefore, potential of leaf litter, especially of aromatic plants, acting as inhibitory source also needs extensive study.

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